Title Development of an optimized sampling regime for the determination of the effects of bioaerosols on health

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DEVELOPMENT OF AN OPTIMISED SAMPLING REGIME FOR THE DETERMINATION OF THE EFFECTS OF BIOAEROSOLS ON HEALTH

by

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ABSTRACT

Bioaerosols vary in size between particle types which affects their airborne properties, influencing the type of sampler that can be used when trying to detect them. Despite this, there is no standardised protocol for measuring bioaerosols.

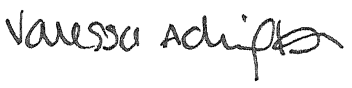
Sampling experiments were performed in indoor environments with low concentrations of bioaerosols (office and domestic residences) to evaluate the sampling efficiencies of the Andersen 6-stage viable impactor, Omega AIRTEST viable sampler, AGI-30 liquid impinger and filter samplers. These sampling methods were evaluated both individually and in comparison with each other. The measurement of indoor particulate concentrations using an LN5 laser monitor and surface sampling of indoor dust were also performed.

The most appropriate methodologies for use with each sampler are recommended, based on the sampled data from this study. Representative measures of bioaerosol concentrations were achieved that were directly comparable with the other methods but it was concluded that no single sampling method is suitable for comprehensive bioaerosol sampling. The effect of human activity in an indoor environment was found to have a particularly significant effect on measured bioaerosol concentrations.

Preliminary findings from this study show evidence of a dose-response relationship and suggest that there are a larger number of reported health symptoms for environments with higher bioaerosol concentrations. However, further work requires to be done that will allow predictions to be made about the severity of likely health effects according to measured bioaerosol concentrations for a particular environment.

DECLARATION

I declare that this thesis is my own unaided work. It is being submitted for the degree of Master of Philosophy at the University of Bedfordshire. It has not been submitted before for any degree in any other University.



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ABBREVIATIONS

CFU: Colony forming units.

CWMEM: Centre for Waste Management and Environmental Monitoring (University of Luton).

DEFT: Direct Epifluorescence Filter Technique.

DoH: Department of Health.

LPS: Lipopolysaccharide (endotoxin).

LTA: Lipoteichoic acids.

MEA: Malt Extract agar.

MPN: Most probable number.

NA: Nutrient agar.

PDA: Potato Dextrose agar.

PG: Peptidoglycan.

PM10: Particulate matter with an aerodynamic diameter equal to or less than 10 μm.

PM2.5: Particulate matter with an aerodynamic diameter equal to or less than 2.5 μm.

PM1: Particulate matter with an aerodynamic diameter equal to or less than 1 μm.

TSA: Tryptone Soy agar.

TSP: Total suspended particulates.

UV: Ultra-violet.

CONTENTS

Table of Figures

Table of Tables

1 AIMS AND OBJECTIVES

There is little consistency in the bioaerosol sampling methodologies that have been used in the numerous different studies that have been carried out to date. This study will examine several important factors affecting the effectiveness of bioaerosol samplers and the results will lead to an increased understanding of the performance of bioaerosol samplers and contribute to an optimised regime being developed.

The main aims of this study were:

- To review the air sampling methods currently available for the monitoring of bioaerosols in indoor environments.
- To compare the performance of commonly used bioaerosol samplers by examination of collection efficiencies and microbial recovery rates both in the laboratory and in the field.
- To determine the most appropriate air sampling methods for indoor environments including offices and domestic housing and develop an optimised sampling strategy for sampling of indoor bioaerosols.
- To determine the effect of human activity and environmental disturbance on measured bioaerosol concentrations in indoor environments.

- To investigate the relationship between measured bioaerosol concentrations in domestic environments and the health effects experienced by people in those environments, with a view to being able to predict likely health effects.

The bioaerosol samplers selected for use in this study were the Andersen 6-stage viable sampler, the AGI-30 all glass impinger, the Omega AIRTEST viable sampler and polycarbonate filter samplers. Additionally, a LN5 laser particle counter was employed to measure total airborne particulate concentrations. To achieve these aims several separate studies will be performed.

1. The sampling efficiency of the Andersen sampler will be tested to compare the effects of collection media, sample times and incubation temperatures.
2. The sampling efficiency of the Omega AIRTEST will be compared to that of the Andersen sampler and measured bioaerosol concentrations will be compared against total airborne particulate concentrations.
3. The measured bioaerosol concentrations collected by the AGI-30 impinger and filter samples will be compared against simultaneous results from the Andersen sampler.
4. Airborne particulate concentrations will be measured in an indoor environment using the LN5 laser sampler and the effect of human activity on particulate and bioaerosol concentrations examined.

5. The comparability of results between different samplers will be examined with a view to recommending a standard sampling regime that will achieve reproducible and meaningful results.
6. The measured bioaerosol concentrations in a set of domestic residences will be compared against reported health effects, obtained from questionnaire data from a separate ongoing study.

The main hypotheses tested include:

- Plastic plates induce electrostatic effects, reducing the number of colony counts obtained when using the Andersen sampler (Andersen, 1958).
- Increased sampling time decreases the Andersen sampler's collection efficiency (Folmsbee, 2000).
- Growth medium and incubation temperature have an effect on bioaerosol sampling.
- There are significant daily variations in environmental conditions that effect bioaerosol sampling results.
- There is a relationship between airborne particulate and bioaerosol concentrations.
- Carpeted floors and human occupants are a likely source of indoor bioaerosols.
- Exposure to indoor bioaerosols will have an effect on human health.

This work aims to fill some of the gaps found in the field of bioaerosol research. It is well documented that there is a lack of a standard sampling method for bioaerosols. In addition, the effects of biological aerosols on health are not fully known and there is an absence of a clear dose-response relationship for bioaerosol exposure.

2 INTRODUCTION

2.1 Bioaerosols

2.1.1 Definition of bioaerosols

An aerosol consists of finely divided material, of variable composition, suspended in air or another gaseous environment (Hirst, 1995). Bioaerosols can therefore be defined as airborne particles of biological origin or activity – meaning that, whether a whole organism, an organism fragment or a biochemically active component, the particles originated from a biological source. Inhalation of bioaerosols can affect human health by a variety of mechanisms, such as infection, allergenicity or toxicity. Bioaerosols can contain bacteria, fungi and viruses, as well as pollens, products of microbial metabolism (proteins, enzymes, endotoxins, mycotoxins, glucans) and particles of zoological origin. Particles range in size from aerodynamic diameters of 0.01 to 100 μm, where viruses are the smallest particle and pollen the largest. These variations in size affect the specific airborne properties of each particle type, which in turn influences the type of sampler that should be used when attempting to detect or quantify such particles (Lacey & Dutkiewicz, 1994).

2.1.2 Where are bioaerosols found?

Bioaerosols are found in many occupational environments including medical areas, microbiology and biotechnology laboratories, agriculture, fibreglass manufacturers, sewage treatment works, composting and animal slaughterhouses. Bioaerosols are also found in less contaminated indoor environments such as offices and houses (Teeuw *et al.*, 1994; Saraf *et al.*, 1997, Chao *et al.*, 2002). Researchers are becoming increasingly aware that bioaerosols are a common occurrence in everyday environments and that exposure to biological aerosols can pose a significant health risk to many occupational and domestic groups (Macher & First, 1984; DeCosemo *et al.*, 1991; Li *et al.*, 1999).

2.2 Survival of microorganisms in air

Air is not a suitable medium for the growth of microorganisms. It is lacking in water, nutrients, substrates for growth etc. resulting in an environment high in environmental stresses. In general, Gram-positive bacteria are more resistant to dry conditions than Gram-negative organisms due to their cell wall being thicker and more rigid, conferring better drought resistance (Section 2.7.1). Fungi are generally found in terrestrial environments and both sexual and asexual fungal spores tend to be resistant to drying, heating, freezing and some chemicals.

2.3 Bioaerosol components important in human health

As well as whole organisms, organism fragments and cellular components from living or dead cells can have a major effect on human health. Several of the most important components, with regard to documented health effects are detailed.

2.3.1 Endotoxin (lipopolysaccharide)

Endotoxin is a well studied bioaerosol component that is documented to have a range of effects on human health. Endotoxin is associated only with Gram-negative bacteria and is a major component of their outer cell membrane. It is continually being shed into the environment making it ubiquitous in our surroundings. Chemically, endotoxins are lipopolysaccharide (LPS) molecules comprising a lipid portion and a long-chain polysaccharide component. The lipid component of the molecule, Lipid A, is predominantly associated with its characteristic toxic activity and is distinctly different to other lipids commonly found in biological membranes (Duchaine *et al.*, 2001). The LPS molecule as a whole is not toxic when incorporated in the cell outer membrane. However, when it is released from the cell wall, when for example the cell multiplies, dies or is lysed, the Lipid A component evokes an immune response.

Endotoxin exposure is common in many occupational environments and studies have been carried out to investigate levels of exposure and its effects in a range of environments. These include, fibreglass manufacturing plants (Walters *et al*, 1994; Milton *et al.*, 1996a, 1996b), livestock buildings (Seedorf *et al.*, 1998) and cotton processing plants (Kennedy *et al.*, 1987), as well as clinical studies on

volunteer subjects (Michel *et al.*, 1997; Kline *et al.*, 1999). As well as the inhalation of endotoxin, studies have also been carried out to examine the effects of instillation directly into the lung (O'Grady *et al.*, 2001) and administering endotoxin intravenously (Suffredini *et al.*, 1992).

Several studies have also been undertaken to look at levels of airborne endotoxin in domestic environments (Douwes *et al.*, 2000; Heinrich *et al.*, 2001; Park *et al.*, 2001). A cross-sectional study carried out by Michel (1999), to test whether the inhalation of endotoxin in house dust would worsen asthmatic symptoms such as bronchial inflammation, found that endotoxin from house dust was responsible for a significant reduction in lung function in asthmatic individuals. It was concluded that endotoxin, as a pro-inflammatory substance, had a clinical effect on the lung function and medical status of asthmatic subjects. Rizzo *et al.* (1997) also found evidence that house dust endotoxin worsened the symptoms of childhood asthma.

2.3.2 Peptidoglycan

Peptidoglycan (PG) is a cross-linked heteropolymer of glycan strands responsible for the mechanical strength and rigidity of bacterial cell walls, thus assisting in maintaining their shape. It consists of β-1-4 linked chains of alternating residues of *N*-acetyl glucosamine and *N*-acetyl muramic acid. Although present in both Gram-negative and Gram-positive bacteria, PG occurs in much greater amounts in Gram-positive bacteria forming the major component of the cell wall. In Gram-positive bacteria this is approximately 90% of the cell wall weight (20-80 nm thick) compared to Gram-negative bacteria where the PG layer is much thinner,

comprising only 15-20% of the cell wall. Peptidoglycan has been referred to as "Gram-positive bacterial endotoxin" (Szponar & Larsson, 2001) and, in a similar manner to Gram-negative endotoxin (lipopolysaccharide), can be a powerful stimulator of the human immune system (Liu *et al.*, 2001). *In vivo*, PG or partial structures of PG can reproduce the common signs and symptoms of bacterial infection including fever, inflammation and lymphocyte activation. Zhiping *et al.* (1996) studied PG as part of a study into inhaled swine-house dust. There is, however, little detailed knowledge about the effects on health of peptidoglycan inhalation.

2.3.3 Muramic acid

N-acetyl muramic acid is an amino sugar found in PG and therefore is uniquely associated with bacterial cell walls. For this reason, it can be measured as an indicator of total bacterial biomass, detectable in viable and non-viable bacteria as well as cell wall fragments (Szponar &Larsson, 2001, Laitinen *et al.*, 2001).

2.3.4 Lipoteichoic acids

In addition to PG, the cell walls of Gram-positive bacteria also contain lipoteichoic acids (LTAs) which, like peptidoglycan, are thought to cause a systemic inflammatory response in humans (Cleveland *et al.*, 1996; Ginsburg, 2002). Like peptidoglycan, lipoteichoic acid has also been referred to as the Gram-positive equivalent of endotoxin and resembles lipopolysaccharide, both structurally and functionally (Ginsburg, 2002). Lipoteichoic acids are composed of glycerol-containing acidic polysaccharides called teichoic acids that are bound

to the membrane lipids of Gram-positive bacteria. They extend through the peptidoglycan layer and are exposed on the surface of the cell. They have a phosphate-linked backbone of glycerol or ribitol. Instead of the lipid A in LPS, lipoteichoic acid has a diacylglycerol lipid moiety. Like Gram-negative bacteria, Gram-positive organisms are becoming increasingly apparent as a causal agent of septic shock and multiple organ failure, with the likely cell components affecting this ability being PG and LTA. However, not all Gram-positive species are pathogenic in humans. Several studies have proposed that lipoteichoic acid may act in synergy with peptidoglycan to induce sepsis (de Kimpe *et al.*, 1995; Kengatharan *et al.*, 1998). It is hypothesised that the specific structure of the LTA determines the pathogenicity of a particular bacterium, while PG amplifies an LTA-induced response. However, the work carried out to investigate the action of lipoteichoic acids have generally been *in vivo* and *in vitro* studies. Little, if anything, is known about the inhalation effects of LTA.

2.3.5 Fungal fragments

As well as spores, filamentous fungal colonies release mycelial fragments, which when aerosolised contribute to the fungal load of indoor air (Górny *et al.*, 2002). These fragments are known to be immunologically reactive and can induce allergic reactions to the same degree, if not more than, fungal spores (Aukrust *et al.*, 1985; Fadel *et al.*, 1992). In a review by Górny (2004) it is stated that this important component of bioaerosols is not detectable or measurable by most of the commonly used bioaerosol samplers.

2.3.6 β(1→3)-glucans

Glucans are glucose polymers found in fungal cell walls and most higher plants, and are also secreted from certain bacteria. In most fungi they are present as β(1→3)-glucans, a chain of glucose compounds joined mainly by β(1→3)-polyglucoside linkages. β(1→3)-glucan is therefore commonly used as a marker of fungal exposure in studies of indoor air and bioaerosol exposure (Chew *et al.*, 2001). Glucans are considered to be non-allergenic in humans but are well known to induce non-specific inflammatory reactions, often, in cases of bioaerosol exposure, resulting in respiratory symptoms. In a study investigating sick building syndrome (where ill health is associated with occupancy of a particular room or building) Rylander *et al.* (1992) found eye irritation, dry coughing and itchy skin to be related to levels of airborne β(1→3)-glucan in office buildings. Wan and Li (1999) detected a strong association between lethargy and fatigue of office workers and airborne fungal glucan concentrations. In terms of respiratory effects, Douwes *et al.* (2000) found that an 18-fold increase in β(1→3)-glucan levels measured in living room carpet dust (μg per m^2 carpet) increased the variability in peak expiratory flow (PEF) of asthmatic children. Similar results have been reported by Andriessen *et al.* (1998), associating childhood respiratory symptoms (measured as PEF variability) with reported fungal growth in the home. The results of experimental exposures in human challenge tests (Rylander, 1996) also indicate the potential for glucans to have an inflammatory effect.

Despite the studies that have been carried out in this field, valid research in this area is limited (Douwes, 2005) so there is restricted knowledge on the potential

respiratory health effects related to β(1→3)-glucan inhalation. As so often described in the field of bioaerosol sampling this is due, in part, to a lack of specific, sensitive and cost-effective methods for monitoring environmental glucans.

2.3.7 Mycotoxins

A mycotoxin can be defined as a "mould-produced secondary metabolite that is injurious to vertebrates upon ingestion, inhalation or dermal contact" (Robbins *et al.*, 2000) and covers a variety of compounds produced by many species of mould. A range of mycotoxins has been found in fungal mycelia and spores as well as on growth matrices e.g. damp wood, plaster, foodstuffs. Generally, mycotoxins are large, complex, non-volatile molecules which can bind directly with DNA and RNA, and can interfere with cellular respiration or carbohydrate and lipid metabolism. A single mould species may be capable of producing more than one mycotoxin and, in contrast, the same mycotoxin may be produced by several different moulds. Because of this, several mycotoxins may be present in one environment making it impossible to tell which, if any, is having an effect. Even if a known toxin-producing fungus is present, the mycotoxin may not be produced in that environment as very specific environmental conditions are often required. Aflatoxins are a commonly documented example of mycotoxins and are produced by several species of *Aspergillus*. They are commonly found associated with maize and groundnuts (Moss, 1998), particularly during post-harvest storage. Ochratoxins are another group of mycotoxins, produced by both *Penicillium* and *Aspergillus* species, which are known to be immunosuppressive and probably

carcinogenic (Kuiper-Goodman & Scott, 1989). *Alternaria*, commonly found in indoor environments with moisture-damaged timber, is another fungal species associated with mycotoxin production and produces around thirty-five toxic secondary metabolites. *Stachybotrys chartarum* is another common contaminant in damp buildings and is documented to produce numerous compounds that are likely to affect the indoor environment (Brasel *et al*, 2005). Moss (1998) stated that although there is a lack of severe acute effects associated with human exposure to mycotoxins, chronic effects caused by their immunosuppressivity and carcinogenicity should be regarded seriously. In contrast, however, a review by Robbins *et al.* (2000) found that health effects in humans can be associated with the inhalation of, or direct contact with, high levels of mycotoxin or mycotoxin-producing moulds. It was concluded, however, that mycotoxins are generally present in low concentrations and it is only rarely that concentrations are high enough to cause a dramatic and clearly linked health effect.

2.4 Health effects of bioaerosols

The adverse effects of bioaerosols on health are well documented and Douwes *et al.* (2003) provide a comprehensive review on the subject. Viable pathogenic microorganisms can cause hazards to human health but microbial cell wall components themselves may also cause serious health effects. For example, endotoxin from Gram-negative bacteria and (1→3)-β-D-glucan from moulds have inflammatory and adjuvant properties and are said to cause toxic pneumonitis, airway inflammation and allergic asthma (Rylander *et al.*, 1992). Several studies (Milton *et al.*, 1995, 1996b) have reported reduced lung function and severe flu-

like symptoms in fibreglass manufacturers exposed to endotoxin from Gram-negative bacteria in washwater mists. Systemic effects such as joint and muscle pain have also been experienced. Research suggests that asthma symptoms may be exacerbated by exposure to bioaerosols. In addition, sick building syndrome and organic dust toxic syndrome are two examples of the consequential health effects of bioaerosol exposure. However, although knowledge is increasing, the effects of biological aerosols on health are not fully known and there is an absence of a clear dose-response relationship for bioaerosol exposure (Verhoeff & Burge, 1997; Douwes *et al.*, 2003; Fung & Hughson, 2003).

2.4.1 Allergic reactions

Bioaerosols, containing viable or non-viable organisms, are capable of causing allergic reactions and producing symptoms in sensitive individuals (Jacob *et al.*, 2002). These may include wheezing, coughing, itchy eyes and nose, sore throat and sinus congestion. For an individual to experience an allergic reaction they require to have been previously exposed to the microorganism and symptoms can occur after re-exposure to even very small amounts of the microbe antigens.

2.4.2 Asthma

Asthma can be caused by several factors, including an allergy to microorganisms. For example, *Alternaria* and *Penicillium* spores can provoke immediate and delayed-onset asthma in sensitized persons. A study was carried out to examine the association between asthma symptoms and severity and indoor bioaerosols (Ross *et al.*, 2000). Indoor concentrations of viable mould spores, bacteria and

dust-mite allergens were measured in forty-four homes and health questionnaires were used to characterise asthma severity in residents. A strong association was found between the concentration of total bacteria and Gram-negative bacteria and asthma-related visits to the hospital emergency room. No significant relationship, however, was found between adverse respiratory effects and indoor fungal concentrations. It is unlikely that meaningful conclusions can be drawn from these results. The authors found a high degree of variation in indoor spore concentrations, not only between different houses, but over time in the same house. This compares with the low variability seen in bacterial concentrations, possibly providing an explanation for the stronger association of asthma symptoms and severity with bacteria than with fungal spores. Inhaled endotoxin is reported to cause "airway symptomatology" and a worsening of asthma symptoms and severity (Kline *et al.*, 1999). A recent study of 2552 house dust samples demonstrated that household endotoxin exposure, from floors and bedding, is a significant risk factor for increased asthma prevalence (Thorne *et al.*, 2005).

2.4.3 Organic dust toxic syndrome (toxic pneumonitis)

Organic dust toxic syndrome (ODTS) is an acute inflammation of the respiratory system which has been attributed to the inhalation of bioaerosols. The onset of symptoms, which are often 'flu-like in nature, usually occurs following a single exposure to large concentrations of highly contaminated dust. In contrast to an allergic reaction, however, ODTS occurs as result of the direct toxicity of fungal exposure rather than being directly immune mediated. Zhiping *et al.* (1996)

examined the effect on various aspects of health, such as lung function and body temperature, of inhaling peptidoglycan. Both lipopolysaccharide (endotoxin) and peptidoglycan were associated with an increase in interleukin-6, a cytokine produced as a result of inflammation. An increase in body temperature correlated with exposure to peptidoglycan, while lipopolysaccharide appeared to contribute to general malaise and shivering as well as negative changes in lung function. It was concluded that rather than all the observed health effects being attributable to endotoxin, "multiple agents" from both Gram-positive and Gram-negative bacteria were responsible for the observed airway inflammation and related health effects in this study.

2.4.4 Sick building syndrome

In recent years, it has become accepted that working in modern office buildings may have a deleterious effect on health (London Hazards Centre, 1990). Workers commonly present symptoms ranging from lethargy and headaches to eye, nose and skin irritation (Burge *et al.*, 1987, Vincent *et al.*, 1997), all of which fall under the heading of "sick building syndrome". Airborne microorganisms are thought to be one possible cause of sick building syndrome and although much of the available data is contradictory, several investigations have provided evidence for this. Teeuw *et al.* (1994), for example, found that in buildings where symptoms such as dry, itchy eyes, blocked nose or sore throat were prevalent, a corresponding high concentration of Gram-negative bacteria and airborne endotoxin was also present. A study by Rylander *et al.* (1992) also suggested that glucan was responsible for the symptoms seen in cases of sick building syndrome.

2.5 Legislation

There is no standardised monitoring protocol for measuring bioaerosols (DeCosemo *et al.*, 1991), despite the fact that there is a legal requirement for air quality to be monitored. For example, under the Control of Substances Hazardous to Health Regulations (COSHH) 2002 (as amended), the main purpose of which is to protect people against health risks from hazardous substances at work, biological agents and bioaerosols are classed as 'substances hazardous to health' and employers must monitor exposure, assess the consequent risks and introduce controls where necessary. A health-based occupational exposure limit for endotoxin has been recommended by a Dutch expert committee (Dutch Expert Committee on Occupational Standards, 1997). "No effect levels" for inhaled endotoxin have been calculated ranging from 9 – 180 $ng.m^{-3}$ (Heederik & Douwes, 1997). This range of values is seen due to the fact that different methodologies have been used in these studies, leading to large differences in results. Based upon these results, an occupational exposure limit of 4.5 $ng.m^{-3}$ (50 Endotoxin $Units.m^{-3}$) has been recommended for airborne endotoxin. As yet, the UK has no such legislation. Previous research has failed to determine which sampling device is best for assessing exposure to endotoxin in a particular environment and how performance compares between available samplers. This is partly due to observed inconsistencies in evaluations of bioaerosol sampler performance (Macher, 1997).

2.6 Types of bioaerosol sampler

Bioaerosol monitoring includes the measurement of both viable and non-viable microorganisms in the air. Viable microorganisms are defined as those "capable of reproducing under appropriate conditions" (Singleton & Sainsbury, 1996), i.e. they are metabolically active. Viable microorganisms can be subdivided into two groups – culturable and nonculturable. Culturable microorganisms are capable of reproducing under laboratory conditions whereas nonculturable ones are not. Nonculturable microorganisms will not reproduce in the laboratory because of intracellular stresses or conditions being non-conducive to growth. Viable bioaerosol sampling, therefore, results in an underestimation of bioaerosol concentration as only those culturable microorganisms are enumerated and identified. Non-viable microorganisms, by definition, are not capable of reproduction under standard conditions and can therefore not be enumerated or identified by growth dependent methods such as impaction onto agar (although there are occasions when non-viable microorganisms can be resuscitated). However, non-viable cells are usually collected onto filters and subsequently counted and identified using traditional microbiology and microscopy or by molecular or immunochemical techniques. Different samplers are likely to give different results (Eduard, 1997). An ideal sampler would be one which is most efficient at recovering all bioaerosols from a tested environment and that allows subsequent performance of all required analyses.

For the collection of bioaerosols in occupational settings, commercially available samplers based on the principles of inertial impaction, liquid impaction and

filtration are generally regarded as appropriate. The occupational environments where bioaerosol sampling has been performed have commonly included: pig houses (Eduard *et al.*, 1990, Zhiping *et al.*, 1996); wastewater treatments (Gillespie *et al.*, 1981); metal working workshops (Kriebel *et al.*, 1997); solid waste handling facilities (Crook *et al.*, 1987); fibreglass manufacture (Milton *et al.*, 1995, Milton *et al.*, 1996a, Milton *et al.*, 1996b, Walters *et al.*, 1994); mouldy building remediation (Rautiala *et al.*, 1998) and microbiology laboratories (Smid *et al.*, 1989). Many of these environments have been regarded as highly contaminated.

For indoor domestic environments, where bioaerosol levels are generally lower than in occupational environments, culture methods are the most commonly used technique for assessing the microbial content of airborne dust. This provides a quantitative measure of the number of viable organisms. It has been said, however, that the majority of airborne microorganisms are non-culturable and it is generally accepted that only 0.1 – 10% of total microorganisms in environmental samples are culturable (Krahmer *et al.*, 1998, Szponar & Larsson, 2001). In addition to the Andersen sampler and AGI-30 liquid impinger, the following methods are available for aerobiological sampling in indoor environments: 0.4 μm pore polycarbonate Nuclepore membrane filters; 0.8 μm pore cellulose ester membrane filters; portable volumetric sieve samplers; the Reuter centrifugal air sampler; surface air samplers and slit-to-agar samplers. The environments in which these have been used include house dust samples from vacuuming carpet (Saraf *et al.*, 1997) and air sampling in office buildings (Teeuw *et al.*, 1994).

To obtain a measure of a sampler's bioefficiency (performance), laboratory and field comparisons with reference samplers can be carried out. A reference sampler is a well characterised, standard sampler against which others are compared, the most common ones being the Andersen viable sampler and the AGI-30 liquid impinger.

2.6.1 Andersen sampler

The Andersen 6-stage viable particle sampler (Figure 2.1) is a multi-orifice cascade impactor designed for use with agar-filled Petri dishes.

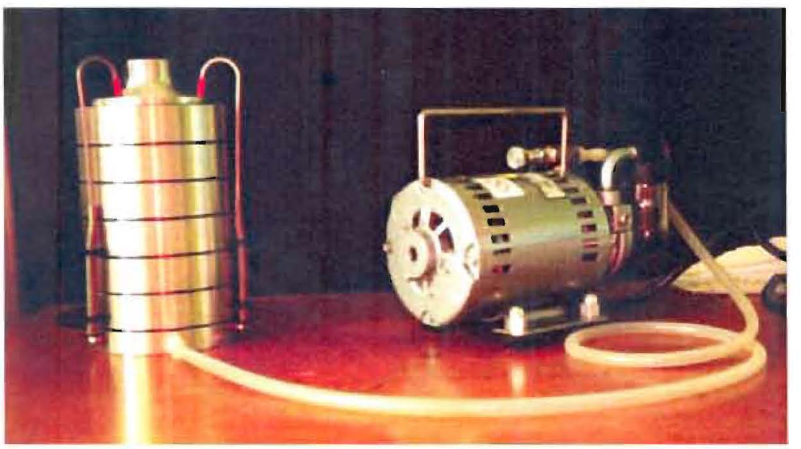


Figure 2.1: The Andersen viable microbial sampler, connected to vacuum pump

Air is drawn through a series of six perforated aluminium plates, each with 400 holes that sequentially decrease in diameter with each plate. The sampler is held together by three spring clamps gasketed with O-ring seals, with an agar

collection plate placed on each stage behind each aluminium plate. Air is drawn through the sampler at a flow rate of 28.3 L min^{-1}, impacting airborne particles from multiple air jets on to the agar surface. Any particles smaller than the cut-off of each stage follow the air stream round the edge of the agar-filled Petri dish to the next stage. Particles are separated into six size fractions, representing the different levels of the human respiratory system. Each stage and the particle sizes it captures are detailed in Table 2.1.

Table 2.1: Andersen sampler stages and equivalent levels of the human respiratory tract

Stage	Particle size cut-off (μm)	Equivalent level of respiratory tract
1	7 and above	Mouth and nose
2	4.7 – 7	Pharynx
3	3.3 – 4.7	Trachea and primary bronchi
4	2.1 – 3.3	Secondary bronchi
5	1.1 – 2.1	Terminal bronchi
6	0.65 – 1.1	Alveoli

2.6.1.1 Collection plates

The Andersen sampler is a standard bioaerosol sampler used throughout industry and research as a reference against which other bioaerosol samplers can be compared. It provides counts of viable cells and particle size distributions by impacting onto agar plates. The instruction manual and manufacturer's handbook supplied with the sampler state that glass collection plates should be used.

Andersen (1958) also discusses this, stating that investigations into the use of glass, aluminium or plastic plates revealed that, on average, plastic plates yielded counts of approximately 20% less than those achieved with glass or aluminium plates. Aluminium plates are not used as their opacity "made the observation and counting of colonies difficult" (Andersen, 1958). Despite these findings, most investigators use commonly available plastic plates instead of the recommended specialist glass plates, without acknowledging the possible discrepancy that this might cause.

2.6.2 AGI-30 liquid impinger

The AGI-30 sampler is a high velocity liquid impinger. It consists of a liquid-containing, glass cylinder attached to a vacuum pump (Figure 2.2).

Figure 2.2: AGI-30 all-glass liquid impinger

Air is drawn through the liquid, impinging any particles onto the bottom surface of the cylinder and trapping them in the liquid. A dilution of this liquid is then plated out onto agar and following incubation, any colonies are counted using standard procedures. Selective or non-selective media may be used. When using the AGI-30 impinger, the type and volume of collection fluid, sample length and holding time and temperature are important factors to be considered (Brachman *et al*, 1964).

Terzieva *et al.* (1996) compared this plate count detection method with staining techniques and size spectrometry. It was found that a high proportion of cells are damaged during collection by impingement, but use of a non-selective medium such as TSA achieved high counts as most of the injured cells recovered.

2.6.3 Filter samplers

Filtration is the most common method used for the sampling of non-biological airborne particles and for this reason there is a wide range of appropriate sampling devices available. The most commonly used filters for bioaerosol sampling are polycarbonate membrane filters which collect particles on their surface rather than embedding them within the filter structure (Crook, 1995b). Filtration is a non-inertial technique and methods are therefore less dependent upon particle size. There are several disadvantages in using filters for bioaerosol collection. These include: the large volumes of air being drawn over the filter surface during sampling often cause dehydration effects, reducing the viability of the collected

organisms; and the fact that samples require subsequent analysis, so further handling is needed and incomplete recovery of the sample is common.

2.6.4 Omega AirTEST sampler

The Omega AirTEST aero-biocollector sampler (Munro, UK) is a handheld rechargeable viable sampler. This is a relatively new piece of equipment and no data on the characterisation or validity of its sampling efficiency has been found in the literature. A polyoxymethylene sampling screen screws onto the sampler face and particles are impacted on to an agar-filled (25 ml) Petri dish positioned inside the sampler. The sampling screen is a 90 mm plastic cover perforated with 265 holes, each 0.6 mm in diameter, through which air is drawn, allowing the collection of particles greater than 0.3 μm diameter. In contrast to the majority of other samplers, rather than setting the time over which samples are to be taken, the desired sample volume is programmed in to the Omega sampler. The sampler then runs, stopping automatically after the required sample volume has been reached. The sampler operates at a flow rate of 100 $l.min^{-1}$ (as compared with 28.3 $l.min^{-1}$ for the Andersen sampler) allowing shorter sampling times than generally used with more recognised samplers.

2.6.5 Slit-to-agar sampler

The slit-to-agar sampler draws air at high speed through narrow slits onto a rotating agar plate, spatially separating the airborne microorganisms for ease of counting and identification (Bourdillon *et al.*, 1941). It is not as commonly used

as some of the previously described samplers as it is documented to be more efficient at sampling pollen and large fungal spores than bacteria and smaller spores, which tend to be under-represented when the slit-to-agar sampler is used (Crook, 1995a). It is described here for information only and was not used in this study.

2.6.6 Negretti LN5 laser sampler

The human respiratory tract can be described as an aerodynamic classifying system for airborne particulates. The effect that airborne particles can have on respiratory health is, not surprisingly, related to their size and, therefore, their ability to penetrate deep into the respiratory system. In contrast to the previously described methods, the Negretti LN5 laser sampler provides data on the overall particulate content of the air, rather than just the biological content. This sampler provides a minute by minute record of the numbers of total airborne particulates and those within three size ranges. These are: PM10 particles, PM2.5 particles and PM1 particles, which are particles with aerodynamic diameters equal to or less than 10 μm, 2.5 μm and 1 μm, respectively. These particle sizes can be related to the approximate size ranges for general groups of microorganisms.

The sampler uses a laser nephelometer to measure the size and concentration of airborne particles and the data is processed to give simultaneous particle measurements. A laser light source provides a beam of light which is passed through the stream of airborne particles. A light-sensing device at right angles to the incident light beam directly measures the scattered light. An internal filter

allows gravimetric analysis of the particles and can also be analysed for bioaerosol content. Microorganisms collected on the filter can be enumerated using microscopy and staining methods or, alternatively, the endotoxin or glucan content of the sample can be determined.

2.6.7 Surface sampling

A number of studies have been carried out that have included surface sampling of indoor floors and carpet. As with many of the methods used in bioaerosol sampling there is no standard and reproducible methodology. Many studies have been carried out to collect microbiological particles from indoor surfaces using methods originally developed for the recovery of lead, pesticides and other chemicals and dust mite allergen. Studies have used vacuum cleaners of various type and size or standardised dust collectors (Leese *et al.*, 1997; Wickens *et al.*, 2004) to collect dust samples from various sizes of sample area covered with various types of material. Lehtonen *et al.* (1993) and Macher (2001b) both used domestic vacuum cleaners to collect dust samples from residential environments. The dust was suspended in solution and dilutions of this plated on to agar for subsequent growth and identification of microbiological content. In contrast, Horner *et al* (2004) used a direct plating method. Sieved vacuumed dust from floor surfaces was sprinkled directly onto agar plates for the determination of culturable fungi in house dust. Petersen *et al.* (1992) also used a vacuum cleaner fitted with a gel collection tape to analyse general particulate contamination on floor surfaces. Rather than using culture-based methods to analyse surface samples, Portnoy *et al.* (2001) measured allergen concentrations in dust samples

collected using domestic vacuum cleaners using immunoassays for several fungal species.

2.7 Detection methods and identification

Following the actual sampling of the airborne microorganisms it is necessary to assess for example, the number of total cells, number of viable cells and types of organism that are collected. Several methods are commonly used for the detection of microorganisms. These include colony counting, microscopy (including UV and fluorescence microscopy), ATP (Adenosine Tri-Phosphate) bioluminescence, chemiluminescence, immunoassay, polymerase chain reaction (PCR) and biochemistry-based methods. DeCosemo *et al.* (1991) state that before any of these detection techniques can be accepted as standard methods, comparative studies in controlled conditions are required.

2.7.1 Gram staining to classify Gram-positive and Gram-negative bacteria

The Gram staining method is based on the cellular structure of bacterial cells. Gram-positive and Gram-negative cells differ in their relative proportions of peptidoglycan (Section 2.3.2) and muramic acid (Section 2.3.3) which are major components of the cell wall. Additionally, Gram-negative cells have an outer wall containing endotoxin (Section 2.3.1), the Gram-positive equivalent of which are lipoteichoic acids (Section 2.3.4), which are exposed on the outer surface of the cell. The Gram-negative cell wall has a complex multilayered structure, while Gram-positive bacteria tend to have a much thicker cell wall that is comprised

primarily of peptidoglycan (Section 2.3.2). During Gram-staining an insoluble complex of crystal violet and iodine forms inside the cell. This complex is extracted by alcohol from Gram-negative but not from Gram-positive bacteria. The thick peptidoglycan cell wall of Gram-positive cells causes them to become dehydrated by the alcohol. This causes the pores in the wall to close, preventing the crystal violet-iodine complex from escaping. In Gram-negative cells the alcohol easily passes through the outer membrane and the very thin peptidoglycan layer, allowing the stain to escape.

Following a successful Gram stain, further identification techniques are required to identify the genera or species of bacteria isolated.

2.7.2 API identification system for bacteria and yeasts

The API identification system provides test kits for the identification of Gram-negative and Gram-positive bacteria and yeasts. The API system is a biochemistry-based detection method that enables a series of biochemical tests to be performed on a culture of an unidentified microorganism, in order to establish its identity. The organism in question is isolated and grown on a medium and at a temperature specific to the type of organism for which the API kit is designed. For example, API STAPH is an identification system for the genera *Staphylococcus* and *Micrococcus*, where organisms are subcultured onto blood agar and incubated at 35-37°C – the optimum growth conditions required for these organisms. The results of the tests are given in the form of a profile number

which is decoded to give the identity of the microorganism. The available kits are documented to cover most bacterial groups and more than 550 species.

Specific testing kits are available for the identification of:

1. Gram-negative bacteria.
   - Enterobacteriaceae and non-fermenting Gram-negative bacteria, Gram-negative non-Enterobactiaceae, Gram-negative bacilli and Campylobacter species.
2. Gram-positive bacteria.
   - Clinical staphylococci and micrococci, streptococci and micrococci and related genera, Corynebacteria and Listeria species.
3. Anaerobes.
4. Yeasts.
5. Lactobacillus.
6. Bacillus.

Limitations of this technique, however, include the fact that the correct test kit must be used on the appropriate organism type. Cultured organisms must be correctly Gram-stained and consequently cultured on the correct growth medium in order that the most suitable test kit is selected. The use of an inappropriate test kit is likely to result in an incorrect identification being made.

2.7.3 UV and fluorescence microscopy

The majority of microbial cells that can be observed under the microscope are as a result of them being 'viable but non-culturable' i.e. unable to form colonies on a plate. As previously described (Section 2.3) any health effects of bioaerosols are

more likely to be caused by microbial cell components or products than viable microorganisms. For this reason, the total microbial load, including both viable and non-viable cells, should be measured rather than only those that are culturable (Eduard & Heederik, 1998). Fluorescence microscopy is a non-culture based method that allows recognition and enumeration of microorganisms individually, amongst other particles or as "complex aggregates". It is common for fluorescent stains to stain non-microbial particles as well as the microorganisms of interest so shape is a vital factor in the identification of bacterial and fungal particles. Often, however, small bacteria are close in size to the optical resolution of the light microscope, making their shape difficult to characterise (Eduard *et al.*, 2001).

2.7.4 Acridine orange staining

Acridine orange is a fluorochrome or dye which binds to nucleic acids of bacterial, fungal and other cells, either in their native or denatured state. It is a fluorescent planar molecule that inserts (intercalates) into the nucleic acid molecule, between adjacent base pairs. This can be used in fluorescence microscopy to distinguish between double-stranded DNA (which fluoresces green) and single-stranded nucleic acids (which fluoresce orange-red) and determine whether a cell can be classed as viable or non-viable.

2.8 Considerations when developing a bioaerosol sampling strategy

There are several criteria that need to be considered when carrying out bioaerosol monitoring. The behaviour of the bioaerosol itself is governed by the principles

of gravitation, electromagnetism, turbulence and diffusion. Sampling methodologies can be affected by the bioaerosol of interest and its anticipated air concentration (i.e. the likely microbial load). Bioaerosol "viability, identification and quantitation" (Walton & Vincent, 1998) are each areas that require careful consideration when developing a sampling strategy. Ambient environmental conditions such as air temperature, speed, pressure, moisture content and turbulence may also influence the use of a particular sampler. In addition, practicalities such as cost or nuisance factors caused by the noise or wearing of a sampler are worth considering.

2.8.1 The need for a standard sampling protocol

The previous sections of this chapter have detailed the numerous different collection and analysis methods for the study of bioaerosols. With so many techniques available it is inevitable that different studies will be carried out using different approaches, often related to their suitability for the environment being tested. As previously stated, however, this makes the comparison of results from each study difficult, as it is not always known how one sampler or method of identification compares with another.

Several authors have carried out studies to assess the comparability of results obtained when using different bioaerosol samplers. Jensen *et al.* (1992) compared the collection efficiency of each of eight samplers against a standard AGI-30 glass impinger. Experiments were carried out using a test aerosol of known free bacteria, rather than a 'natural' aerosol in the field. Out of the eight samplers

tested, the 6- and 1-stage Andersen samplers and the AGI-30 impinger compared most favourably. Four bioaerosol samplers (including a newly developed high volume cyclonic liquid impinger) were also compared by Cage *et al.* (1996) in an outdoor environment, measuring naturally-occurring bioaerosols. Comparable results were found between the AGI-30 and the high volume impingers, with low collection efficiencies of liquid impingers being a cited disadvantage.

2.8.2 The effect of sampling conditions

Existing studies using the Andersen sampler have tended to concentrate on the use of only one growth medium, temperature or sampling time so the effect of each of these variables should be investigated further.

2.8.2.1 Sample time

Sampling can often present problems in occupational environments where levels of airborne microorganisms (especially fungal and actinomycete spores) are high. Traditional methods, such as the slit sampler and Andersen sampler, which rely on the deposition of particles onto a surface, are now restricted in their areas of use as plates become overloaded quickly. Shorter sampling times are therefore required, resulting in a high degree of variation between samples. This is due to the fact that bioaerosol concentrations vary over time, therefore the shorter the sampling time the greater the probability that the result will be unrepresentative. Folmsbee *et al.* (2000) have examined the effect of sampling time on the total efficiency of the Andersen sampler (modified 2-stage). Sampling of viable bacteria was carried out near a composting centre over times ranging from one to six minutes. Total

counts per m^3 of air were calculated as the sum of counts from only stages 1 and 2. It was concluded that the optimal sample time was 2 minutes, as counts decreased with all subsequent times to 6 minutes.

2.8.2.2 Growth medium and incubation temperature

Nutrients are required for the successful culture of collected microorganisms and different culture media vary in their composition and, therefore, the organisms they are more conducive to the growth of. Many studies use just a single medium and incubation temperature. For example, Lembke *et al.* (1981) carried out air sampling at solid-waste handling facilities using only one sheep blood agar and all incubations were at 30°C. In contrast, Li & Lin (1999) examined the sampling performance of impactor samplers using only TSA plates, incubated at 37°C.

A study by Morring *et al.* (1983) involved a statistical comparison of the effect of four fungal growth media on the number of colony forming units detected when sampling with the 6-stage Andersen sampler. Media were either specific for moulds or contained antibiotics to prevent bacterial growth. The four media examined were: Sabouraud Dextrose agar, Littman Oxgall agar, Inhibitory Mould agar and Rose-Bengal-streptomycin agar. Significant variation was found between the different media with Rose-Bengal-streptomycin agar being selected as the optimum medium for "broad spectrum aeromycological sampling" (Morring *et al.*, 1983).

The incubation temperature selected for the culture of bioaerosol samples is a particularly important variable. It is likely that the temperature selected for the incubation of samples will determine not only the numbers but also the species of viable microorganisms 'detected' in a sample. For instance, organisms that are pathogenic in humans (e.g. *Staphylococcus* spp, *Streptococcus* spp. and *Candida albicans*) are likely to have an optimum growth temperature of 37°C, the normal human body temperature. Species that are commonly found in environmental samples will tend to grow better at 25°C than 30°C or 37°C. Different incubation temperatures are therefore likely to select particular groups of microorganisms, which may differ in their ability to cause health effects.

In order to provide an element of consistency between different studies, a comprehensive investigation is therefore required to examine the effects of different sampling times, growth media and incubation temperatures on the efficiency of the Andersen sampler.

2.8.2.3 Sampler height and orientation

Work by Micallef *et al.* (1999) describes vertical concentration gradients of particulate matter in indoor environments, where smaller particles are more evenly distributed throughout the air than heavier particles which tend to settle more quickly. This study, however, was concerned with total airborne particulates of particular size ranges, rather than biological particles in particular. There is scant mention, if any, in the literature regarding the effect of sampler height on the efficiency of a sampler's bioaerosol collection. It would, however, be prudent to

consider that when examining the effect of bioaerosols on health a sampler height that represents a person's breathing zone is likely to generate the most useful results.

2.8.3 Effects of environmental conditions on bioaerosol sampling

It would be reasonable to assume that house characteristics play a vital role in determining the airborne levels of bacterial and fungal particles in domestic environments. Factors such as building age, temperature, humidity, visible damp or mould growth etc. are all regarded as capable of having an effect on indoor bioaerosol concentrations and compositions. It is important to be aware of any abnormal environmental conditions that may occur during sampling, raising the possibility that a representative picture of normal exposure is not gained. Often, in sampling studies, these housing characteristics are determined through the use of a questionnaire administered to the household resident. This data is frequently used to predict indoor levels of exposure to aeroallergens, despite concerns that the evaluation of many characteristics is very subjective (e.g. dampness, severity of mould growth) and that no objective measurements of fungal levels tend to be made. Ren *et al.* (2001) evaluated the accuracy with which questionnaire-determined residential characteristics could 'predict' measured indoor fungal concentrations. In addition, the relationship between fungi in indoor air and house characteristics was examined.

2.8.4 Effect of human activity on bioaerosol sampling results

Very few studies have been carried out to examine the effect of human activity and environmental disturbance on indoor bioaerosol concentrations. Several studies have examined the effect of vacuuming on dust, microbial or endotoxin concentrations (Roberts *et al.*, 1999, Bellanti *et al.*, 2000). These studies however, have concentrated more on examining reduction effects on the surface concentrations of dust, rather than on looking for an increase in airborne dust concentrations as result of the vacuuming.

A study by Buttner and Stetzenbach (1993), however, measured the sampling efficiencies of four bioaerosol samplers and the effect of human activity on air sampling results. Sampling was carried out in an experimental room designed to replicate an indoor residential environment. *Penicillium chrysogenum* spores were introduced at a known concentration ($10^5/m^3$ air) through an air supply duct and allowed to settle, contaminating the wall-to-wall carpet with spores. Sampling was carried out using 6-stage Andersen samplers, surface air system (SAS) samplers, depositional samplers and a Burkhard spore trap in parallel with an aerodynamic particle sizer (APS). A researcher walked in a set pattern for 1 minute prior to sampling to represent human activity. A further part of the study involved replacing the walking with vacuuming the carpet for 2 minutes. At moderate levels of contamination (10^5 spores/m^3) walking and vacuuming caused a significant increase in airborne spore concentrations (10^4 spores/m^3) compared with undisturbed air samples (10^3 spores/m^3). It was concluded that where carpet

is a source of indoor microorganisms, air sampling alone without parallel surface sampling may be unlikely to accurately reflect microbial contamination.

A later study (Parat *et al.*, 1999) examined the relationship between airborne bacterial concentrations and particle counts with regard to human activity. Like Buttner and Stetzenbach's earlier work (1993) the sampling was carried out in experimental rooms with measurements taken over 1 hour after an initial period of disturbance. An extension of the study was performed at sites in occupied office buildings, measuring natural variations in bacterial and particle counts cause by normal activity and movement. Variations in bacterial concentration were reflected by corresponding variations in particle count but it was concluded that particle counting should not replace culture techniques for measuring the concentration of airborne microorganisms as no absolute relationship can be established between the two.

2.9 Relationship between indoor and outdoor particles

Much work has been carried out to examine the relationship between indoor and outdoor airborne particulate concentrations, examining not only biological particles but also organic and inorganic substances including combustion particles, recondensed metal vapours and crystalline material. Morawska *et al.* (2001) investigated the relationship between total indoor and outdoor particulate concentrations for residential houses. Evidence was found suggesting a relationship between the particulate concentrations outside and those inside, when the house was under "normal" ventilation rates where the air exchange rate was

around 2-5 h^{-1}. However, no definitive relationship allowing the prediction of indoor particles based on those outside could be established for a standard set of environmental conditions.

It has been found by several authors (Wickman *et al.*, 1992; Garret *et al.*, 1997) that the concentration of fungal particles in outdoor air has an important influence on the levels of fungi found in indoor environments. As well as work examining the prevalence of fungi, a study by Dharmage *et al.* (1999) also investigated the effect of residential characteristics on fungal concentrations in homes in Melbourne, Australia. Questionnaire data supplied information on house characteristics. Samples were taken from both floor surfaces and the air and analysed for ergosterol, a fungal membrane lipid (used as an indicator of total fungal biomass) and total viable fungal counts, respectively. Seasonal fluctuations, similar to those seen in outdoor environments, were measured indoors with total fungal concentrations lowest in winter and highest during summer and autumn. The indoor prevalence and seasonal patterns observed for several fungal species, including *Cladosporium* and *Alternaria*, concurred with those measured outdoors.

Another Australian study (Garrett *et al.*, 1998) tested the association between various environmental factors and fungal spore concentrations. The results showed a significant correlation between outdoor fungal concentrations and indoor concentrations of both total and viable spores. It can be concluded however that, despite these similarities between indoor and outdoor

measurements, those fungi found in indoor environments are not entirely composed of those from outdoors (Dharmage *et al.*, 1999).

The air in indoor environments, specifically of occupied buildings, contains higher concentrations and a greater variety of species of bacteria than outdoor air (Otten & Burge, 1999). Common indoor bacterial isolates tend to be of human origin and include Gram-positive *Micrococcus*, *Staphylococcus*, *Cornebacteria* and *Streptococcus* species (Burge, 1995). Outdoors, the majority of bacterial species present are those released from the surfaces of living plants or soil. They include both Gram-negative and Gram-positive species such as *Actinomycetes*, *Bacillus*, *Micrococcus*, *Pseudomonas* and *Staphylococcus*.

2.10 Damp houses, mould growth and human health

There is much evidence of the relationship between damp housing and indoor mould growth. In addition there has been much documented research on the effects of living in damp housing on residents' health.

There is a public awareness that there is a direct effect of living in a damp mouldy home on the respiratory health of the occupant(s). Articles in the general press frequently support this. For example, an article by Crompton in The Times (UK edition), 2002 describes various research showing links between dampness in the home and illnesses ranging from respiratory problems and allergies to cardiovascular disease and rheumatic pain. Scientific research addressing this

issue is also well documented (Garret *et al.*, 1998; Thorn *et al.*, 2001; Dharmage *et al.*, 2002).

2.11 The need for further research and a method that will allow the prediction of health effects from measured bioaerosol concentrations

Further research is needed throughout the field of bioaerosol sampling. Comparative studies of sampling methods and detection techniques are required and an attempt made to standardise bioaerosol collection methods (DeCosemo *et al.*, 1991; Fung & Hughson, 2003). There is also a lack of data available that allows researchers to causally relate specific indoor exposures to symptoms. There is therefore a requirement for methods that will allow a more accurate assessment of bioaerosol exposure as well as accurately representing likely health effects experienced (Verhoeff & Burge, 1997; Douwes *et al.*, 2003).

3 MATERIALS AND METHODS

3.1 Development of sampling techniques

Before a final sampling regime could be designed it was necessary to develop the best and most appropriate method for each of the different samplers. The samplers and techniques being investigated included:

a) The Andersen sampler, for air samples.

b) The Omega AirTEST sampler, for air samples.

c) The AGI-30 all-glass liquid impinger, for air samples.

d) Filter and pumps, for air samples.

e) The Negretti LN5 laser dust monitor, for air samples.

f) The modified Dustbuster® vacuum cleaner, for surface dust samples.

3.1.1 Optimisation of Andersen sampler method

3.1.1.1 The effect of using glass or plastic plates on the efficiency of the Andersen microbial sampler

Data from the manufacturer of the Andersen sampler suggests that plastic agar plates are unsuitable for use with the 6-stage Andersen sampler. The collection efficiency of the six-stage Andersen sampler when using standard plastic agar plates was compared to that when using the custom-made glass plates supplied

with the sampler, to test for any differences, possibly attributable to electrostatic effects from the plastic plates.

Before air sampling was carried out, the vacuum pump for the Andersen sampler was calibrated and the flow rate adjusted to the standard 28.3 $l.min^{-1}$ using a Platon Gapmeter (C.T. Platon, Hampshire, UK). Before each sample run, the sampler itself was cleaned and swabbed with 70% iso-propyl alcohol to remove any surface contamination which may have affected the results.

A standard set of conditions was used to compare the performance of the Andersen sampler when using different collection plate types. Comparative sets of glass and plastic plates were run for 10-minute intervals in an area of relatively low contamination - an indoor office environment. The office had a floor area of approximately 100 m^2 and was arranged in an open-plan form, to accommodate around 10 people. Each collection plate contained 27 ml Nutrient agar (OXOID, UK), in accordance with the Andersen sampler manufacturer's instructions. Each set of glass and plastic plates was run on the same day to minimise the influence of daily variations in environmental conditions. All samples were incubated for 72 h at 30°C. Total colony counts and distributions were performed for each plate and the results recorded. All colony counts were transformed, as standard, using the positive hole correction factor (Section 3.1.1.2), with results recorded as colony forming units (cfu). These counts were then expressed in the standard form of colony forming units per cubic metre of air ($cfu.m^{-3}$), to give a figure for bioaerosol concentration.

Eight replicate sets of glass and plastic plates were run to account for the effects of environmental variation. General weather conditions were recorded on each sample day (e.g. warm/cold, dry/wet, calm/windy, sunny/cloudy).

In addition to total efficiency, the distribution of colonies on each plate, as a ratio of those in the outer 5 mm to those in the remaining plate area, was also examined. Variation in the numbers of colonies near the outer edges of the plates would be indicative of any electrostatic effects that the plastic plates may have had on sampling efficiency. For the last four replicate sets of plates, colonies within 5 mm of the edge of the plate were counted separately in order to allow a comparison of the proportion of colonies in the outer 5mm of each Petri dish with the proportion of the total plate this area made up.

3.1.1.1.1 Conversion of colony counts using positive hole correction

Counts achieved using the Andersen sampler must be transformed to account for the fact that as the number of particles sampled increases, the probability of the next particle passing through a previously 'unused' hole in the aluminium plate decreases. Viable counts are corrected using the "positive hole" conversion table, that is included in the Andersen sampler instruction manual, to allow for multiple deposition of particles through the holes of each aluminium stage. Numbers of colony forming units are then converted to concentrations by dividing the number of cfu by the volume of air sampled. These values were directly comparable to those corrected values in MPN/m^3, calculated for the Omega AirTEST sampler (Section 3.5.4.1).

3.1.1.2 The effect of sampling time, growth medium and incubation temperature on the total efficiency of the Andersen microbial sampler

Experiments were carried out to examine the effects of growth medium, incubation temperature and sampling time on total microbial counts obtained using the Andersen sampler. Sampling was carried out in the same office environment as previously (section 3.1). A total of four media (Nutrient agar (NA), Malt Extract agar (MEA), Tryptone Soy agar (TSA) and Potato Dextrose agar (PDA)), three incubation temperatures (25°C, 30°C and 37°C) and two sampling times (2 minutes and 10 minutes) were compared.

All growth media were supplied by OXOID, Basingstoke, UK (code numbers: NA (CM3), MEA (CM59), TSA (CM131), PDA (CM 139)) and were made up according to the manufacturer's instructions. Plastic collection plates were used for all experiments. The vacuum pump and Andersen sampler were prepared for use as described in Section 3.1.1.1.

Where possible, at least two replicate sets of each growth medium were run on the same day to limit inter-experimental variation which may have been caused by daily fluctuations in environmental conditions, such as wind direction and strength, humidity, sunlight, disturbance due to room usage etc. General weather conditions were recorded on each sample day. Samples were taken for each of the two sample times and incubated at each of the three temperatures (25°C, 30°C and 37°C) giving six treatments in all for each medium. Experiments were repeated

until every combination of medium, sampling time, and incubation temperature had been replicated three times.

All plates were incubated for 72 h and colony counts recorded for each sample, transforming data using the positive hole correction as previously described (Section 3.1.1.1.1).

3.1.1.3 The effect of sampler height on the total efficiency of the Andersen microbial sampler

The effect of sampler height on the bioaerosol concentrations measured by the Andersen sampler was examined. A 6-stage Andersen sampler was used to take 9 samples for each of two different sampling heights (at floor level and 1.5 m above floor level) onto both nutrient and malt extract agars. The height of 1.5 m was chosen to be approximate to that of the breathing zone of a person of average height. Samples were taken in each of 6 different domestic houses and incubated at 37°C for 72 h. The total numbers of samples taken were 54 on NA at each of the two test heights and 54 on MEA at each height. Following incubation of the sample plates, colony counts were converted into colony forming units (cfu) per cubic metre of air sampled, and the bioaerosol concentrations measured at each height compared to look for an effect of sampler height.

3.1.1.4 The effect of wind speed and direction on bioaerosol sampling results

The effects of local wind speed and direction on the bioaerosol concentrations recorded by both the Andersen sampler and Omega sampler were examined. An Andersen sampler and an Omega sampler were used to take 24 and 48 samples, respectively, in an indoor office environment (Section 3.1.1.1), over fourteen different sampling days. Following incubation of the sample plates from each instrument, colony counts were converted into colony forming units (cfu) or most probable number [of colonies] (mpn) per cubic metre of air sampled, for the Andersen and Omega samplers, respectively. Wind speed and direction data was collected from Rothamstead Experimental Research Station, Harpenden, Hertfordshire for those days on which bioaerosol sampling was carried out. Each speed and direction measurement was recorded as the average for that day over 8 hours, from 9am to 5pm. The office in which the bioaerosol sampling was carried out had large south-facing windows, around the edges of which, at times, it was possible to feel a draught from outside. Wind direction recordings were compared against the aspect of the room windows, looking for a relationship between southerly winds and increased bioaerosol concentrations recorded on days that this was the prevailing wind direction.

3.1.2 Comparison of the sampling efficiencies of the Omega AirTEST and Andersen sampler

The Omega AirTEST sampler was provided on loan from Munro Environmental (UK) for evaluation purposes and was not available for use beyond the method development stage. Preliminary sampling experiments were carried out to

compare the sampling efficiency of the Omega sampler with that of the LN5 particulate monitor. Samples were taken in an office environment, shared by up to 10 people (Section 3.1.1.1), collecting air samples on to both NA and MEA, using standard 90 mm disposable plastic collection plates. In addition, the sampling efficiency of the Omega sampler was then compared against that of the Andersen sampler, a recognised reference sampler. Samples were taken in the same office environment with each sampler running at the same time to allow comparison of results. The LN5 laser monitor ran throughout each sampling period. The Andersen sampler was run for 10 minute periods achieving total sample volumes of 283 litres. The Omega sampler was programmed to collect 280 litre samples, which was the nearest equivalent volume achievable with this sampler. All samples were collected onto NA and plates incubated for 72 hours at 30°C. Initial results showed that the sample volume for the Omega sampler was too high for the environment being sampled. Many of the culture plates became overloaded and it was impossible to accurately count the numbers of individual colonies. Further Omega samples were carried out using half the original sample volume (140 litres). All results were converted to a concentration of mpn per m^3 of air sampled, to allow comparisons of the measured concentrations collected by the two viable samplers to be made.

3.1.2.1 Conversion of colony counts

The Omega AirTEST sampler was supplied with conversion tables and a computer program to allow the conversion of colony counts to a most probable number (MPN) of viable particles actually delivered to the agar surface. This was

based on a similar principle to that which the positive hole correction for the Andersen sampler is based (Section 3.1.1.2). The most probable number of microorganisms per cubic metre of air sampled (MPN/m^3) is calculated based upon the volume of air drawn through the sampler and the number of viable colonies which were grown on the culture plate. The value can either be looked up on the conversion table provided, or the value can be entered in to a Microsoft Excel worksheet and the calculation performed automatically. Both techniques result in the same answer. All colony counts from tests performed using the Omega sampler were transformed using this calculation to give a result in MPN/m^3. These values were directly comparable to those corrected values in cfu/m^3, calculated for the Andersen sampler (Section 2.5.1.).

3.1.3 Comparison of the sampling efficiencies of AGI-30 all-glass liquid impinger and the Andersen sampler

Preliminary experiments were carried out using the AGI-30 liquid impinger in parallel with the Andersen sampler to take samples in an indoor office environment. In accordance with the results of a study by Lin *et al.* (1997) an appropriate sample time and liquid volume were chosen that would allow the most effective sample collection. Before use the impinger flask and tube were sterilised by autoclaving at 121°C for 15 minutes. A volume of 20 ml of sterile ¼ strength Ringers solution (collection fluid) was added to the impinger for collection of the bioaerosol particles. The impinger was then connected via a length of flexible tubing to a vacuum pump and air was drawn through the impinger at a flow rate of 12.5 $l.min^{-1}$. Samples initially ran for approximately 10 minutes. Following

sample collection, the impinger fluid was serially diluted and spread plates made of the neat solution and subsequent dilutions down to 10^{-5}. The liquid was plated on to both NA and MEA plates which were then incubated at 30°C for 72 hours. Simultaneous samples were also run using the Andersen sampler, on the same two growth media. Plate counts were then compared for the two different samplers. The results from these initial experiments showed no microorganisms in any of the impinger samples.

Due to the negative impinger sampling results, the next logical step was to confirm that the impinger method was actually effective i.e. that it allowed not only the collection of, but also the survival and growth of, collected viable cells. Sampling was carried out using the impinger with the nozzle placed several millimetres from the spore-producing surface of colonies of both *Aspergillus* and *Penicillium* on culture plates in the laboratory. This was to represent a heavily contaminated environment of known species. As previously, 20 ml of Ringers collection fluid and a sample flow rate of 12.5 $l.min^{-1}$ were used. Samples ran for 10 minutes. Serial dilutions were plated on to NA and MEA and all samples incubated at 30°C for 72 hours. The results confirmed that the sampling method was effective at the collection of viable particles and that the microorganisms survived the collection process to allow successful growth on agar.

After confirming that sampling with the AGI-30 impinger was effective at collecting viable particles the methodology was adjusted to use a sample time of 25 minutes rather than 10 minutes as had been used previously. Further samples

were then taken in parallel with the Andersen sampler, as first described, using a collection time of 25 minutes. This would allow the collection of detectable particles from an indoor environment.

All further use of the AGI-30 all-glass impinger was in accordance with the method developed here. All samples were taken using 20 ml ¼ strength Ringers solution at a flow rate of 12.5 $l.min^{-1}$ for a sampling time of 25 minutes.

3.1.4 Filter samplers for the collection of airborne microbiological particles

A preliminary study was carried out to examine the suitability of polycarbonate membrane filters for the collection of airborne microbial particles, in particular their effectiveness at entrainment of microbial particles and also the ease with which they would allow the removal, by washing, of these particles for subsequent analyses e.g. acridine orange staining.

Isopore™ (37 mm, 0.8 μm pore size) polycarbonate membrane filters (Millipore UK) were placed into a 25 mm 3-piece polythene filter cassette (Millipore UK). Each filter was backed by a supportive absorbent pad (Millipore UK). The filter cassette was connected to a rechargeable vacuum pump (PAS20/S/1, Pascal Scientific Ltd., UK) and air drawn through the filter at a flow rate of 2 $l.min^{-1}$ ± 0.1 $l.min^{-1}$, impacting any airborne particles on to the filter surface. Each sample was run for a sufficient time in which to allow the collection of a significant number of particles for the rest of the analysis i.e. approximately 2 hours. For

control purposes, two replicate filters were run simultaneously, allowing an examination of the reproducibility of the sampling results.

Following each sample run, the surface of each filter was examined using scanning electron microscopy (SEM). Electron micrographs were taken of the surfaces of a) a clean, unused filter, b) a used, soiled filter and c) a used filter, following washing. Depending on the recovery rates achievable for the removal of microbiological particles from the filter surfaces, subsequent analyses would be carried out on the resulting particulate solutions.

3.1.5 Measurement of total airborne particulate concentrations using the Negretti LN5 laser monitor

Total airborne particulate concentrations in the same indoor office environment as previously described (section 3.1.1.1) were measured using the Negretti LN5 Laser sampler. This was run simultaneously with the Andersen sampler to allow a comparison of these two sampling methods and to examine whether total airborne particulate levels accurately reflect the airborne concentrations of viable microorganisms in a particular environment.

The LN5 laser sampler was run over two separate time periods: one covering 2 days and the other over a period of a week, including a weekend. In each case, before sampling began, a 25 mm, 0.4 μm pore size, glass fibre filter was fitted inside the laser sampler. The sampler was then programmed to record environmental data i.e. concentrations of total suspended particulates (TSP),

PM10, PM2.5 and PM1 particles, every minute throughout the sampling period. These results were compared to the numbers of viable colonies collected by the Andersen sampler, during the same sampling period.

3.1.6 Surface sampling using Dustbuster® vacuum cleaner

3.1.6.1 Pilot study to collect dust samples from floor surfaces

Surface sampling was carried out to assess the effectiveness of removing an identifiable microbiological sample from floor dust. Sampling was performed using a hand-held portable 9.6 V Dustbuster® vacuum cleaner (Black and Decker®, UK). The vacuum cleaner was modified slightly to allow the connection of a 15 cm hardened ashless paper filter (Whatman International Ltd, UK). This filter paper was folded into quarters and inserted in the cleaner before the collection bag to allow the collection of dust onto a filter paper. The flow rate of the vacuum cleaner was 900 $l.min^{-1}$. A 0.5 m^2 square wooden frame (quadrat) was constructed in which to vacuum to ensure that the same floor area was vacuumed during each sample. An area of floor was randomly selected and the quadrat placed down to mark the area to be sampled. The selected area was then vacuumed for 2 minutes in a regular pattern designed to cover the entire surface. The soiled filter was then removed and stored in a sealed plastic universal for weighing. Control samples were collected by putting a filter in the vacuum cleaner, switching it on without making contact with a surface and immediately removing the filter for storage.

Problems were encountered when weighing the recovered dust from each sample. In preliminary experiments the collected dust was shaken from the filter in to a plastic universal for weighing. It was not vital to ensure that all the dust was removed from the filter as calculations would be carried out merely on the amount of dust which was diluted out for further analysis. However, it was not possible to weigh the dust in this way as a steady, non-fluctuating value could not be obtained with the balance used (Oxford Analytical Instruments, UK). The method was then modified. Before sampling, each clean filter was folded and sealed in a separate sterile plastic universal. After a period of equilibration, the weight of each filter and its container was recorded. The same measurement was repeated following dust collection, after a similar period of equilibration, allowing the calculation of the weight of total dust collected per sample.

3.1.6.2 Enumeration and identification of microbiological component of dust samples

The viable microbiological component of each dust sample was assessed using culturing techniques. As it was not practicable to remove the dust sample from the filter, 10 ml of sterile Ringers solution was added to the laden filter in the universal. The filter was vibrated for 2 minutes to release the dust into the sample fluid. Once a concentrated suspension had been achieved the sample was serially diluted to a concentration of 10^{-4} by adding 1 ml of the sample to 9 ml of ¼ strength Ringers solution. Spread plates were made using a 0.1 ml volume of each dilution, plated onto both MEA and NA and incubated for 48 hours at both 25°C and 37°C. Colonies were counted and their numbers per volume of air

sampled and morphologies recorded. The commonest species were identified using Gram staining and the API biochemical identification system as described in Section 3.2 or using colony morphologies.

3.2 Identification techniques

3.2.1 Morphological identification and Gram staining of cultured microbial species

Upon successful culture of the collected microorganisms, preliminary identifications were made of the most commonly occurring species, using observational techniques to record the morphological characteristics of each colony. Details that were recorded included:

- colony shape and size,
- colony colour (both on the upper and lower surfaces),
- the surface texture of the colony.

These characteristics were recorded for both fungal and bacterial colonies.

Fungal colonies were further identified using microscopic techniques to examine hyphal structures and spore arrangements.

To aid the identification of bacterial species, and as a prerequisite for carrying out the API biochemical identification detailed in Section 3.2.2, Gram staining of bacterial colonies was also performed. Gram staining is best performed on cells that are 24 – 48 hours old so colonies that were selected for Gram staining were

subcultured on to fresh agar and reincubated to obtain a pure culture. A smear of cells was then flame-fixed to a glass slide and the staining procedure carried out.

Following staining, slides were examined under the microscope, using the oil-immersion objective lens. On completion of successful staining, Gram-positive cells show as purple. Gram-negative cells, in contrast, stain red.

3.2.2 Identification of common airborne isolates using the API identification system

The API identification system (bioMérieux sa, France) was used to make biochemical identifications of the most common bacterial species found during the Andersen sampling of the indoor office environment described in section 3.1. Gram stains of each unknown were carried out to ensure that the appropriate API kits be used for each organism. Each test was then performed according to the manufacturer's instructions and the results recorded. These results were used in addition to the physical characteristics of each colony to make an identification.

3.2.3 Acridine orange staining of filter samples

Once a filter sample had been taken, it was possible to wash the filter to remove any particles adhering to its surface. Acridine orange staining was then performed to allow a measure of the total cell count for that sample to be made using a fluorescence microscope (Direct Epifluorescence Filter Technique – DEFT). In

contrast to culture techniques or biochemical identification where classification is performed on viable cells that have been cultured, acridine orange staining is designed to be performed on both viable and non-viable cells, providing a measure of the total cell count in a sample.

3.2.3.1 Preparation of buffers and solutions

Stock solutions were made up prior to the acridine orange staining procedure being carried out. These were:

a) *Filter wash fluid*

Filter wash fluid was comprised of 1 g bacteriological peptone (code L37, Oxoid, UK), 0.05% (v/v) Tween 80 (BDH Laboratory Supplies, UK) and 20 g myo-inositol (Sigma, UK) dissolved in 1 litre of distilled water. The solution was then autoclaved at 121°C for 15 minutes.

b) *DEFT buffers*

Two buffers of different pH (pH 6.6 and pH 3.0) were prepared using 0.1 M solutions of citric acid and sodium hydroxide. A 0.1 M citric acid solution was made up using 21 g of citric acid powder (Fisher Scientific, UK) per litre of distilled water. Sodium hydroxide powder (Fisons Scientific Equipment, UK) was added to distilled water (4 g/l) to make up a 0.1 M solution.

The pH 6.6 buffer contained 35.5 ml 0.1 M citric acid in 100 ml 0.1 M sodium hydroxide. The buffer of pH 3.0 was composed of 100 ml 0.1 M citric acid and

54 ml of 0.1 M sodium hydroxide. The pH values of each buffer solution were confirmed using a pH meter (Corning model 5, Corning Ltd, UK) and adjusted as necessary.

c) *Acridine orange stain*

Acridine orange stain was freshly prepared each day to preserve its fluorescence. Rather than using the stain in powder form, a ready-prepared acridine orange 10 mg/ml in water solution (Molecular Probes, USA) was used at a concentration of 1 ml per 49 ml of pH 6.6 buffer. A precipitate formed when these two solutions were mixed so the stain was passed through a 0.2 μm syringe filter before being transferred to a plastic universal for temporary storage in the dark.

All stain and buffer solutions were filter sterilised using 25 mm 0.2 μm cellulose acetate membrane syringe filters (Nalgene®, USA) prior to use to maintain sterility and remove any particulate contamination.

3.2.3.2 Staining procedure

Before washing, exposed polycarbonate filters were wetted using 1 ml of filter wash fluid, pipetted into the bottom half of the filter cassette. This wetting process was performed to maximise the volume of extractable fluid that would be present post-washing. Each filter was then washed by adding 5 ml of filter wash fluid to the filter through the top half of the cassette and vibrating for 1 minute. The cassette was then opened and the wash fluid transferred to a plastic universal using a sterile plastic syringe. Any heavily concentrated samples were serially

diluted by adding 1 ml of the sample to 9 ml of ¼ strength Ringers solution (1 Ringers tablet (Oxoid) dissolved in 500 ml of distilled water and autoclaved at 121°C for 15 minutes).

A 25 mm glass vacuum filter holder (Sartorius Ltd., UK) was attached to a vacuum source through a Buchner flask. Each sample was filtered through an Isopore™ 25 mm 0.4 μm pore black polycarbonate membrane filter (Millipore, UK) placed shiny side up on to the sintered glass support in the filtration unit, to separate and collect the cellular component from the wash fluid. A 1 ml volume of the sample was added to the filter and the vacuum applied until the fluid had dispersed/been drawn through and any cells deposited on the filter surface. The vacuum was then released and the filter overlain with 2 ml of the prepared acridine orange solution and left for 5 minutes. The vacuum was then applied as before. Leaving the vacuum on, the filter and any deposits were rinsed with three 1 ml volumes of pH 3.0 buffer followed by 2 x 1 ml of isopropanol/propan-2-ol (BDH, UK). Once the liquid residue had dispersed the vacuum was released and, using forceps, the filter removed. Each filter was air dried for several seconds and then mounted on a glass microscope slide on a drop of Cargille Type FF non-fluorescent immersion oil (Cargille Laboratories, USA). A second drop of immersion oil was applied to the top of the filter before placing on a 25 mm glass cover slip. Slides were then examined by epifluorescence microscopy as described in Section 3.2.3.3.

The initial results obtained from these staining procedures, however, were unsuccessful with very little fluorescence observed under the microscope. The methodology was then modified slightly in an attempt to improve the quality of the results. Future experiments were carried out leaving the filter overlain with acridine orange stain for 10 minutes.

3.2.3.3 Observation and counting of cells using fluorescence microscopy

The DEFT slides of the acridine orange stained cells were observed by epifluorescence microscopy using an Olympus BH2 system fluorescent microscope with appropriate excitation filter set (channel 1 filter set 9, mercury vapour light source with excitation and barrier filter set for acridine orange). It was possible to identify cell types as bacterial or fungal on the basis of their shape and size. Cells were classified as viable or non-viable according to colour, where orange-fluorescing cells were viable and green-fluorescing cells were non-viable. The number of microscope fields counted for each slide was determined by the average number of cells found per field. Any cell or group of cells separated by a distance equal to, or greater than, twice the smallest diameter of the cells nearest each other, was counted as a separate clump. Diplococci and chains of cocci were counted as single clumps. Morphologically different cells closely spaced to each other were counted as separate clumps, even if less than two diameters apart. Table 3.1 shows the counting scheme employed for DEFT slides.

Table 3.1: DEFT slides counting scheme

Average number of cells/field	Number of fields counted
0-10	15
11-25	10
26-50	6
51-75	3
76-100	2
+100	dilute sample and repeat

The cell count per ml of sample was then calculated by multiplying the average number of clumps per microscope field by the microscope factor where,

$$\text{microscope factor} = \frac{\text{area of membrane through which sample filtered } (mm^2)}{\text{microscope field area } (mm^2) \times \text{sample volume (ml)}}.$$

The area of membrane used was calculated using the internal radius of the filter tower and the microscope field area was measured using a standard stage micrometer and eyepiece graticule.

Counts were multiplied by the volume of diluent added to each filter (i.e. 10 ml) to give the total number of cells collected during the whole sampling period. Values were then converted to concentrations of cells per m^3 of air by dividing by the volume of air sampled.

3.3 Variations in indoor airborne microbial and particulate concentrations in an office over time and the effect of human activity

The effect of human activity on indoor airborne particulate and bioaerosol concentrations was examined using the LN5 laser sampler in conjunction with Andersen viable sampler and the Omega AIRTEST sampler. The LN5 laser sampler was run over four separate time periods in an indoor office environment. Each sample period lasted for between 24 and 30 hours. One of these sampling periods started at approximately 7 am in an empty office. Another included a weekend. As previously reported (Section 3.1.5), a 25 mm, 0.4 μm pore size, glass fibre filter was fitted inside the laser sampler before sampling began. The airborne concentrations of TSP, PM10, PM2.5 and PM1 particles were then measured and recorded, every minute throughout the sampling period. These results were compared to the numbers of viable colonies collected by the Andersen and Omega samplers, during the same sampling period.

Where sampling began in an empty office before occupants arrived, care was taken during the setting up of the monitoring equipment not to make any unnecessary movement and access to the room was via paper 'stepping stones' to reduce disturbance of the carpeted floor surface. In each case, all of the equipment was in position and started before any of the normal occupants were present in the room. The LN5 laser sampler then ran over the whole working day to include the influx of people to the office at the start of the day through to the emptying of the room at the end of the day. Viable samples were taken using the Andersen and Omega samplers simultaneously, at five time points throughout the

sample period. Samples were taken on to both NA and MEA for each sampler. The Andersen sampler ran for 10 minutes each time, taking one 10 minute sample on NA, immediately followed by a 10 minute sample on to MEA. The Omega sampler was run during these periods of Andersen sampling. As previously described (Section 3.1.2) the Omega sampler was set to take a sample volume of 140 litres. During one 10 minute run of the Andersen sampler, one NA and one MEA sample were taken with the Omega AIRTEST. Therefore, on each sample day, five NA and five MEA samples were taken using the Andersen sampler and ten samples were taken on each of the same two media using the Omega sampler.

3.4 Investigating the likely source of indoor bioaerosols in an office environment

Surface sampling for settled dust from the carpeted floors of several offices was carried out using a handheld vacuum cleaner, taking samples following the method described in Section 3.1.6. Additional samples were taken from the head and shoulders of those rooms' occupants, where the hair and clothes were vacuumed (n=12 for both floor and people samples). All samples ran for 2 minutes. A 10 ml volume of sterile Ringers solution was added to each sample and following agitation for 2 minutes, 0.1 ml of each solution was then plated on to both NA and MEA and incubated at 30°C for 72 hours. The results were examined to look for a relationship between the numbers and types of organisms isolated from each location. The species of organisms isolated from the floor samples were also examined, relative to those found in the air samples. In addition, four floor and four occupant samples were analysed using the DEFT

technique following staining with acridine orange as previously described (Section 3.2.3). The results for numbers of viable and non-viable bacterial and fungal cells were compared between sample locations.

3.5 Effect of human activity on bioaerosol and total airborne particulate concentrations in domestic accommodation

Sampling was carried out in the bedroom of a first floor flat to look at the effects of different domestic activities on airborne particulate and bioaerosol concentrations. The location in which to carry out the sampling was chosen as a result of the presence of extensive mould on the interior walls. The building had a leaky gutter which had lead to the development of a large damp area down the external wall. The obvious appearance of this damp patch and the growth of moss on the exterior brickwork suggested that this has been a long-standing problem. The bedroom was located above an empty, open garage and was poorly insulated. The room had a concrete floor covered with thin carpet and no underlay. Internally, the bedroom felt cold and damp and there was significant visible mould growth on three walls (Figure 3.1). In addition, there were also areas of visible moisture in droplets on the walls and carpets felt damp to touch. All air sampling in this damp home was carried out in the winter months, between December and February.

Table 3.2: Pattern of activities carried out during bioaerosol sampling.

Activity	Set-up
Undisturbed	Sampler on floor, no people or activity in room.
Bed-making	Sampler on floor, gentle disturbance activities e.g. walking, making beds, opening curtains etc.
Hoovering	Sampler on floor, disturbance caused by vacuuming carpet.

On day one only two undisturbed and two bed-making samples were taken (repeat samples). On day two, six samples were taken – two repeat samples for each of the three activities detailed in Table 3.2. A third set of sampling was carried out running the same experiments in 'reverse order', sampling during the greatest disturbance activity first and in an undisturbed environment last. As on day two, two repeat samples for each of the three activities detailed in Table 3.2 were taken but on this occasion sampling was carried out in parallel with a LN5 laser sampler which ran throughout the sampling period.

In addition to measuring the numbers of particulates and viable microorganisms, the species collected were also examined. A comparison was made between the fungal species found growing on the internal walls of the bedroom and the fungi cultured on the sample plates following air sampling.

3.6 The comparison of domestic bioaerosol concentrations measured using different sampling methods

During this work, there was an ongoing study in CWMEM (Centre for Waste Management and Environmental Monitoring, University of Luton) funded by the Department of Health (DoH) entitled "Consequences for health, and effects of damp on the incidence of airborne microbial cell wall components in the home" (unpublished).

A group of seven damp houses, representing the worst and least affected, was selected for this work from the list of participant houses registered on the DoH-funded study. Each house was then visited once, in December 2000, with the following sampling regime used in each of the houses to compare bioaerosol concentrations measured using different sampling methods.

The aim of these experiments was to collect bioaerosol data that was representative of the normal range of conditions likely to be experienced within each house. All sampling was carried out in the main living room of each house. Viable air samples were taken during periods of stillness and during periods of air disturbance. These disturbances were created using a custom built fan-agitator (Figure 3.2) designed to disturb and resuspend settled dust from the floor surfaces by blowing air downwards on to the floor.

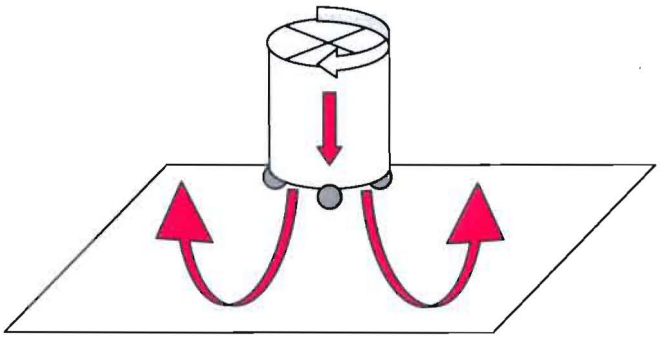


Figure 3.2: Sketch diagram of fan-agitator.

The total sample period for each house was 2 hours, during which there were three 5 minute periods of disturbance. Samples were taken both before and during these times of agitation.

The LN5 laser monitor was run throughout the entire sample period i.e. approximately 2 hours. It was started on arrival in the house and sampled continuously, recording particulate measurements every minute, whilst the other sampler types were used to take samples of the bioaerosols present. Two viable samplers were run in parallel, the Andersen sampler and the Omega AirTEST. Six samples were taken per house using the Andersen sampler – three during normal conditions and one during each of the three periods of disturbance. The Andersen sampler was run for 10 minutes with collection onto NA. In contrast, twelve samples were taken per house using the Omega sampler. Six samples were taken on to both MEA and NA plates for still and disturbed samples. The sample time was two minutes. All samples were incubated at 30°C for 72 hours.

The Andersen sampler was run for 10 minutes. Over that time the Omega sampler was run twice for two minutes, once with NA and once with MEA i.e. two Omega samples were collected at same moment in time as one Andersen sample to allow the comparison of results (Figure 3.3). This was done for three undisturbed and three disturbed samples. Temperature and relative humidity data were also recorded in each house.

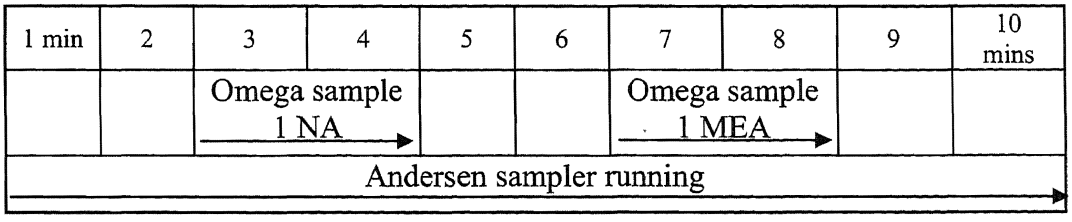


Figure 3.3: Schematic diagram showing sampling regime

3.6.1 Preliminary examination of the relationship between the measured bioaerosol concentrations and the reported health effects experienced by house occupants

The results of the sampling performed in this study, described in Section 3.6, were hoped to be used to complement the ongoing DoH-funded study in CWMEM entitled "Consequences for health, and effects of damp on the incidence of airborne microbial cell wall components in the home". The DoH study included air monitoring in a number of homes using traditional sampling methods as well as the collection of health data, gathered from occupants using a questionnaire (Appendix 1). The questionnaire addressed aspects of the respondents' lives including their occupation, home environment, health status and allergies and

smoking habits. This questionnaire was administered twice by CWMEM to each of the participating house occupants – once during the dry season (April-September) and once in the damp season (October-March). Damp season data was collected after the dry season data. The responses from the questionnaire would be available for analysis and comparison with the air sampling experiments carried out in this study, detailed in Section 3.6. No data resulting from the DoH study has so far been published.

The collected bioaerosol and particulate data for each house was analysed directly against the anonymised questionnaire responses from the occupant(s) of those houses. It was important to ensure the anonymity of the house occupants to maintain the confidentiality assured when the questionnaire was administered. All of the data was coded appropriately for use in the SPSS statistics package and preliminary analyses performed to look for relationships between measured bioaerosol concentrations and reported and measured health effects.

3.7 Comparison of reproducibility of results between different sampling methods

Sampling experiments were carried out in a set of six houses whose occupants had volunteered to participate in the study. The houses were selected to include a range of domestic characteristics including type of heating, presence of pets and resident smokers (Table 3.3).

Table 3.3: House characteristics for six houses used for comparing measured bioaerosol concentration by sampling method.

House number	Heating type	Pets?	Smoker(s)?	Other comments
1	Gas.	3 dogs. 2 cats.	No.	Very dusty. Patches of visible mould.
2	Gas.	No.	No.	Smelt musty, possible mould. Small child in household.
3	Electric / Warm Air.	No.	No.	Severe mould problem in bedroom.
4	Gas.	No.	No.	Asthmatic resident. Very regularly vacuumed.
5	Gas.	Cat.	Yes - 2.	2 children in household.
6	Gas.	3 dogs. 2 cats.	No.	Same occupant as house 1. Newly moved into this house.

The sampling regime used in these six houses, visited in 2001, was different to that previously used in December 2000 (Section 3.5). Indoor measurements were made of airborne particulate concentrations, viable and total airborne microorganisms, the microbiological content of settled carpet dust and indoor temperature and humidity. In addition, on at least one of the three visits, outdoor measurements of viable bioaerosols were made. These samples were taken over 10 minutes using an Andersen sampler, sampling onto both MEA and NA with incubation at 25°C and 37°C.

All six houses were visited three times. In each house, on each visit, the total sampling period was approximately 3.5 – 4 hours to allow time for nine paired samples to be taken using the Andersen sampler. Airborne particulate concentrations were measured using the LN5 laser monitor, which was run continuously throughout the sampling period. Temperature and relative humidity

were recorded every minute using Tinytalk® II data loggers (Gemini Data Loggers (UK) Ltd, Chichester). To enable assessment of the total microbial component of the air, filter samples were taken using plastic cassettes. Each of the two filters was placed at a height of 1.5 m and exposed simultaneously for the entire sampling period, sampling at a flow rate of 2 $l.min^{-1}$ ± 0.1 $l.min^{-1}$. The filters were subsequently washed and acridine orange staining was performed as described in Section 1.2.3.

Andersen sampling was carried out to measure the viable component of indoor bioaerosols in each house. Two Andersen samplers were used in parallel to take measurements that would allow the comparison of different growth media and sampling heights and incubation temperatures. Two set-ups were employed. To compare the effect of sampling height, six paired Andersen samples were taken, three pairs sampling onto NA and three onto MEA with each half of the pair sampling at a different height. The Andersen sampler was placed either on the floor or on a ladder platform at a height of 1.5 m and run for 10 minutes. All plates were incubated at 37°C for 72 h.

In addition, the effects of growth medium and incubation temperature were examined by running another three paired samples. This time, each pair of samples was taken over 10 minutes at a height of 1.5 m – with one sampled onto NA and the other onto MEA. These plates were incubated for 72 h at 25°C.

Viable samples were also taken using the AGI-30 all glass liquid impinger (Ace Glass Inc., USA). Duplicate samples were taken into 20 ml Ringers solution over approximately 25 minutes, at different times in the sampling period. On returning to the laboratory, 0.1 ml volumes of each impinger sample were plated onto NA and MEA and incubated for 72 hours at 25°C and 37°C. It was unnecessary to perform serial dilutions on the collection fluid as previous experiments (Section 1.1.8) had shown very low concentrations of microorganisms to be collected from indoor environments using this type of sampler.

The microbiological content of settled carpet dust was measured by taking samples from the floor surface using the Dustbuster® vacuum cleaner and recording viable microorganism concentrations as described in section 3.7. The experimental setup is shown in Figure 3.4.

Figure 3.4: Experimental set-up to compare the results of different sampling methods.

The results obtained from each sampling technique were then compared against each other to compare the reproducibility of results between the different sampling methods.

Table 3.4 shows the step-wise protocol, as followed during each set of sampling.

Table 3.4: Step-wise protocol for house sampling.

Time (mins)	Action
0	LN5 + dataloggers on
5	filters on
10	load Andersens + switch on (2 heights)
20	switch off Andersens, empty + clean
25	re-load Andersens + switch on (2 heights)
35	switch off Andersens, empty + clean
40	re-load Andersens + switch on (2 heights)
41	impinger 1 on
45	vacuum floor (2min)
50	switch off Andersens, empty + clean
55	re-load Andersens + switch on (2 heights)
60	vacuum floor (2min)
65	switch off Andersens, empty + clean
66	impinger 1 off
70	re-load Andersens + switch on (2 heights)
71	impinger 2 on
75	vacuum floor (2min)
80	switch off Andersens, empty + clean
85	re-load Andersens + switch on (2 heights)
95	switch off Andersens, empty + clean
96	impinger 2 off
100	re-load Andersens + switch on (2 media)
110	switch off Andersens, empty + clean
115	re-load Andersens + switch on (2 media)
125	switch off Andersens, empty + clean
130	re-load Andersens + switch on (2 media)
140	switch off Andersens, empty + clean
150	switch off LN5 + filters, dataloggers stopped

3.8 Statistical analyses

Statistical analyses of all of the data were performed using the SPSS for Windows (Version 12.0.1) computer package (SPSS Inc., USA). It is useful when analysing paired data to be able to compare groups to test the null hypothesis that the two groups come from the same distribution i.e. that they are not statistically different from each other. Where data is parametric, and therefore follows a normal distribution, these comparisons can be made between groups using a *t*-test, which assumes that the data is normally distributed. However, as is inherent in the nature of bioaerosol sampling data, the majority of the results gathered during the residential and office sampling in this study were not normally distributed, and furthermore, they could not be made normal by mathematical transformation. The data was therefore treated as being non-parametric, where there is no assumption for a particular distribution to be followed and data is likely to be skewed. In this case a Mann-Whitney *U* test (the non-parametric equivalent of the *t*-test) is used to compare whether two groups are from the same distribution. However, the basis of a Mann-Whitney test is to rank the data from lowest to highest and the test is performed on the rank scores rather than the data itself. A limitation of this is that some of the detail about the differences between scores is lost, reducing the statistical power of the test. Therefore, using a Mann-Whitney to test the differences between groups of data is more likely to mask a significant effect than a *t*-test. However, non-parametric data must be analysed appropriately so the Mann-Whitney *U* test was the most commonly used statistical test in this study.

When looking for a correlation or association between two non-parametric variables the Spearman's rank correlation was performed, again testing the null hypothesis that there is no association between the two groups of data being analysed.

When looking at the effect of bioaerosol concentrations on reported health symptoms Crosstabs was used to analyse multiple groups of categorised data. One assumption that must be met for Crosstabs to return valid results is that expected values all exceed 5. If any expected values are returned as less than 5 then more data must be collected to increase the proportion of results that falls into each of the categories being analysed.

4 RESULTS

4.1 Optimisation of Andersen sampler method

4.1.1 The effect on efficiency of using glass or plastic plates with the Andersen microbial sampler

After sampling with the Andersen sampler for 10 minutes on Nutrient agar with incubation at 30°C, the mean total colony count achieved using plastic plates was 813 cfu.m^{-3}, which was higher than the 659 cfu.m^{-3} for the custom glass plates (Figure 4.1). This difference, however, was not significant ($p > 0.05$, 2-tailed t-test, Appendix 2) and it was suggested that there was no difference in the collection efficiencies of glass and plastic plates. These results, however, do not concur with the findings of Andersen (1958) who found that plastic plates yield lower colony counts than glass.

There were large differences in colony numbers between the replicate plates sampled on different days, which should be considered. It can be seen in Figure 4.2 that there was much inter-experimental variation in total bioaerosol concentrations when sampling with both plastic and glass plates.

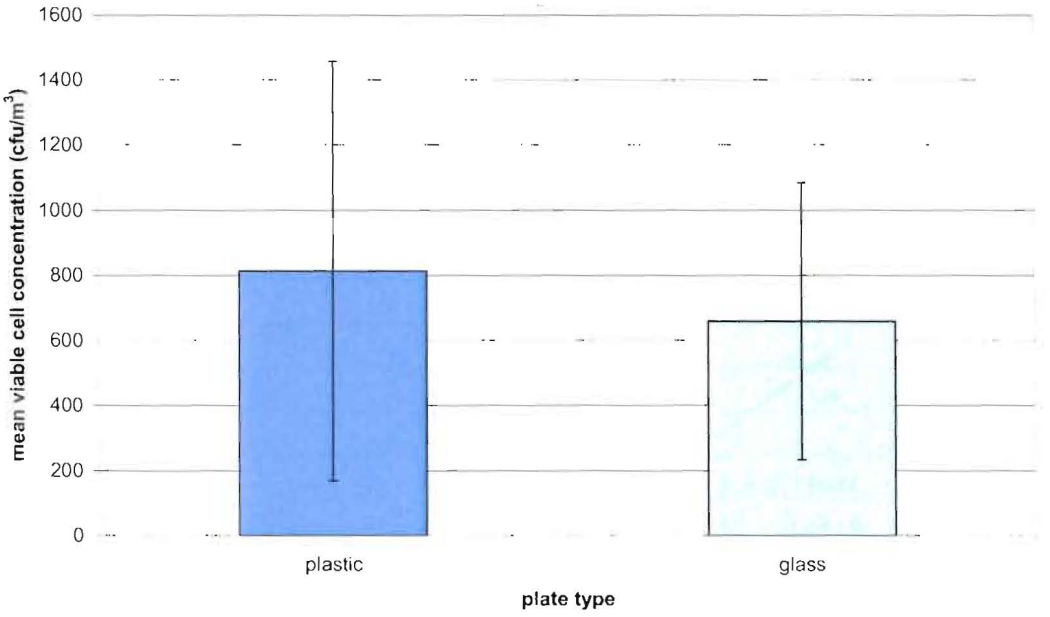


Figure 4.1: Comparison of mean collection efficiencies for the Andersen sampler when using plastic and glass plates (error bars show standard deviation, n = 14).

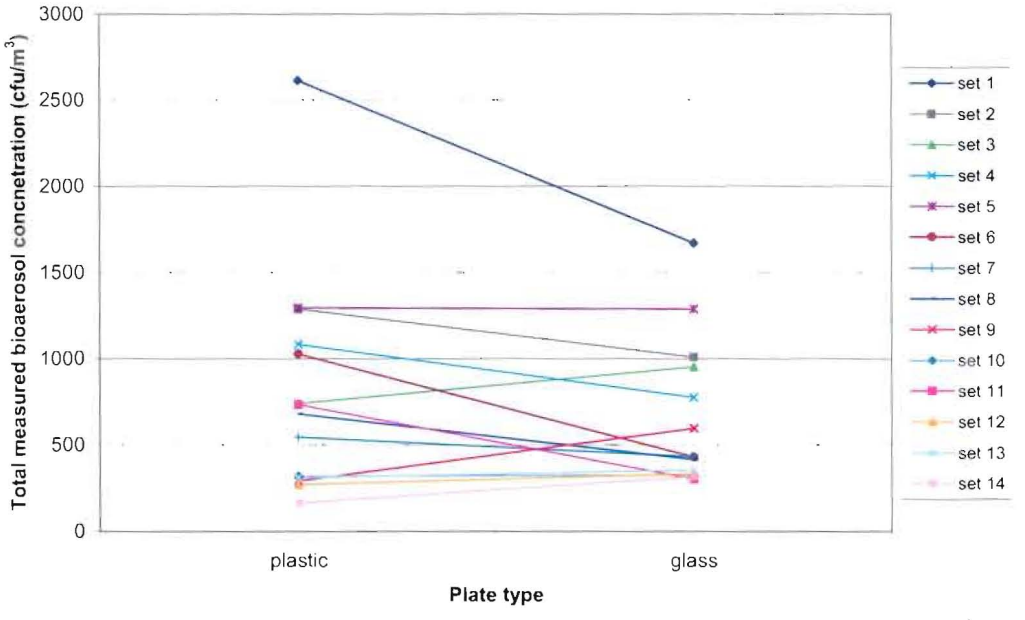


Figure 4.2: Comparison of the collection efficiencies of the Andersen sampler for plastic and glass plates (paired results for each of fourteen replicates).

Figure 4.3 shows the relationship between the counts obtained when using plastic plates and glass plates during simultaneous sampling (correlation coefficient = 0.85).

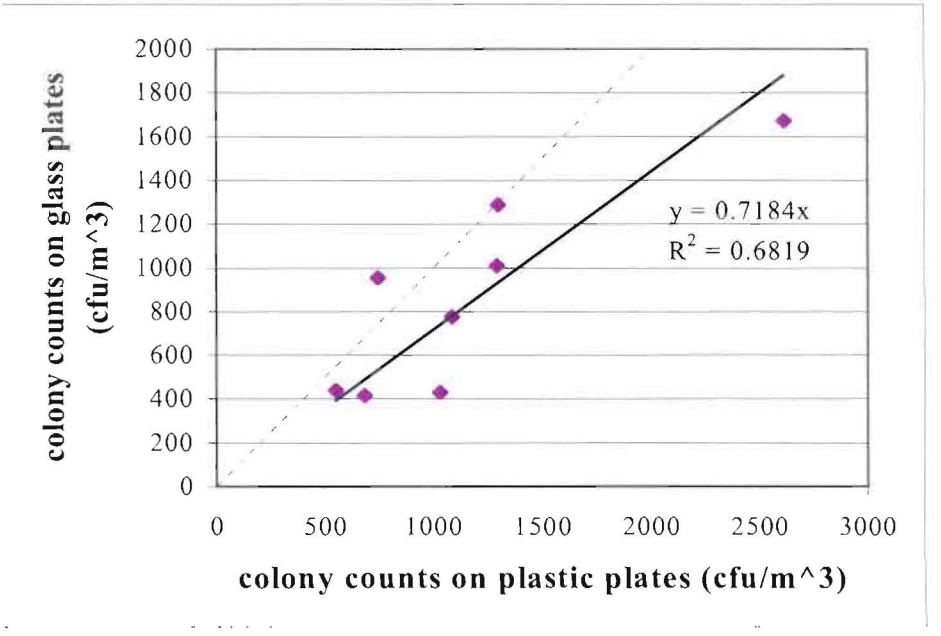


Figure 4.3: Scatter plot showing relationship between counts achieved on plastic plates and those on glass plates (dashed line is 1:1 relationship).

It can be clearly seen that the relationship between the counts achieved using plastic plates and using glass plates deviates from a 1:1 relationship. In fact, it appears that plastic plates have a higher collection efficiency than glass, directly contradicting Andersen (1958). This relationship may be real or may just be caused by the presence of an obvious outlier in the results. Further samples would need to be taken to give a data set large enough to allow statistically valid conclusions to be made.

The mean collection efficiencies of each of the six stages of the Andersen sampler were compared for glass and plastic plates. It can be seen from Figure 4.4 that although there were some differences in colony counts between plastic and glass plates for the six stages, these were not significant (Appendix 2).

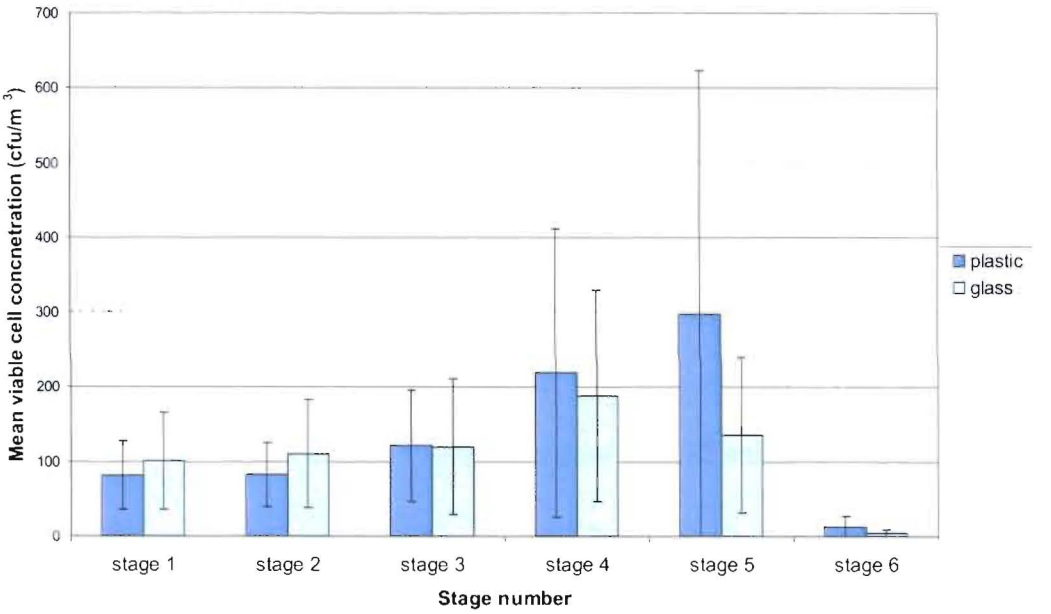


Figure 4.4: A comparison of the mean collection efficiencies of plastic and glass plates for each of the 6 stages of the Andersen sampler (error bars show standard deviation, n = 14).

From the results obtained it can be concluded that the use of plastic plates in the Andersen sampler does not reduce its sampling efficiency, as has been previously stated, and that the effect of daily variation in environmental conditions is far greater than any effect of using plastic plates.

Figures 4.1 and 4.4 show that in this study, for both the Andersen sampler as a whole and its individual stages, the use of plastic plates over the manufacturer's glass plates did not reduce the numbers of colonies detected.

4.1.1.1 Colony distribution on glass and plastic plates

It appeared, on first inspection, that there was a greater distribution of colonies impacted at the meniscus, where the agar met the plate wall, on plastic plates rather than on glass. The distribution of colonies in the outer 5mm of each plate was examined with respect to their distribution on the rest of the plate, to look for evidence of an electrostatic effect associated with using plastic plates. The internal diameters of both the plastic and glass plates were 8.5 cm. Thus, it can be calculated that for such a plate:

Area of circle 4.25 cm radius = 56.72 cm^2.

So, for a circle with the outer 5 mm removed:

Area of circle 3.75 cm radius = 44.12 cm^2.

The area of the plate comprised by this outer 5 mm rim is shown to be 12.60 cm^2, which is equivalent to 22.2 % of the total surface area of plate. This can be compared with the 18.4 % and 17.2 % of total colonies which were found in the outer 5 mm of plastic and glass plates, respectively (Figure 4.5).

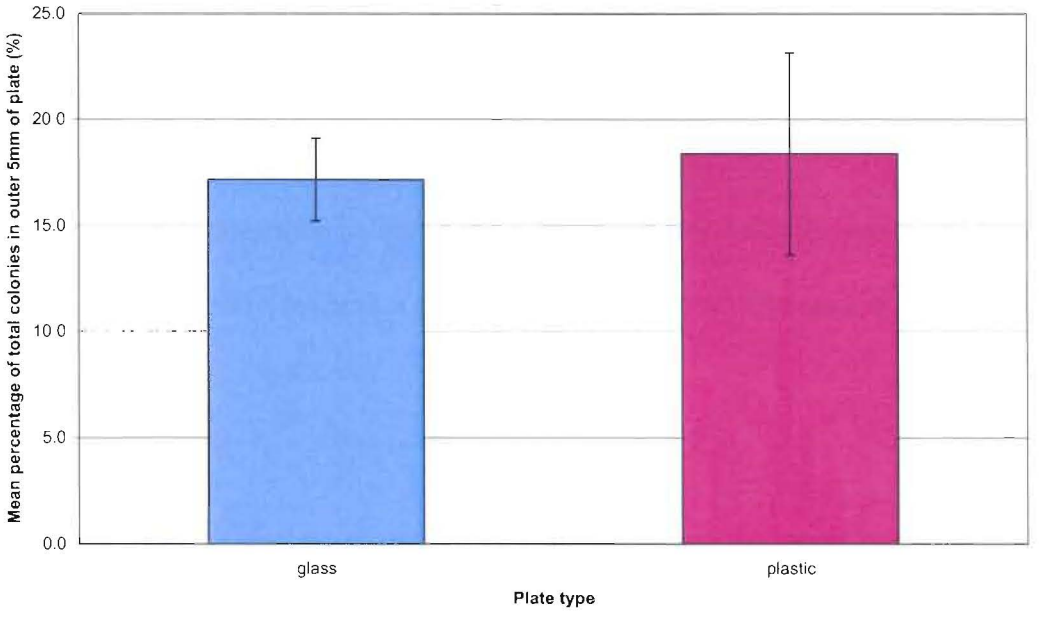


Figure 4.5: Comparison of the distribution of colonies in the outer 5 mm of plastic and glass plates as a percentage of the total colonies (error bars show standard deviation, n=4).

This result shows that there is no significant difference, between plastic and glass plates, in the distribution of colonies over the agar surface ($p > 0.05$, 2-tailed t-test). There were no differences between plastic and glass plates in the proportion of total colonies found at the outer edge. This implies that in addition to there being no evidence of particle retention on the plate exteriors and the sampler walls, there was no retention of particles on the interior surfaces of plates.

As the area of plate comprised by the outer 5 mm rim was equivalent to 22.2% of its total surface area, it would be expected that the proportion of total colonies found in this outer 5 mm would be the same. This was found not to be the case but can be regarded as unimportant as this may merely be a normal consequence

of the design of the sampler, in that particles are not evenly distributed to the very edge of the plates.

The experiments described in Sections 4.1 and 4.1.1 show that there is no negative effect of using plastic collection plates on the collection efficiency of the 6-stage Andersen sampler.

4.1.2 The effect of sampling time on the efficiency of the Andersen microbial sampler

All further investigation of the Andersen sampler collection efficiency was performed using plastic collection plates. The effect of sampling time on total microbial counts obtained using the Andersen sampler was examined. The Andersen sampler was run for two different sampling times – 2 minutes and 10 minutes. Total colony counts were performed for each time across all four growth media (NA, MEA, TSA and PDA) and the concentration of viable cells in each sample calculated (Figure 4.6). Provided the efficiency of the sampler remains constant, sampling for 10 minutes should collect give the same air concentration of microorganisms as sampling for 2 minutes.

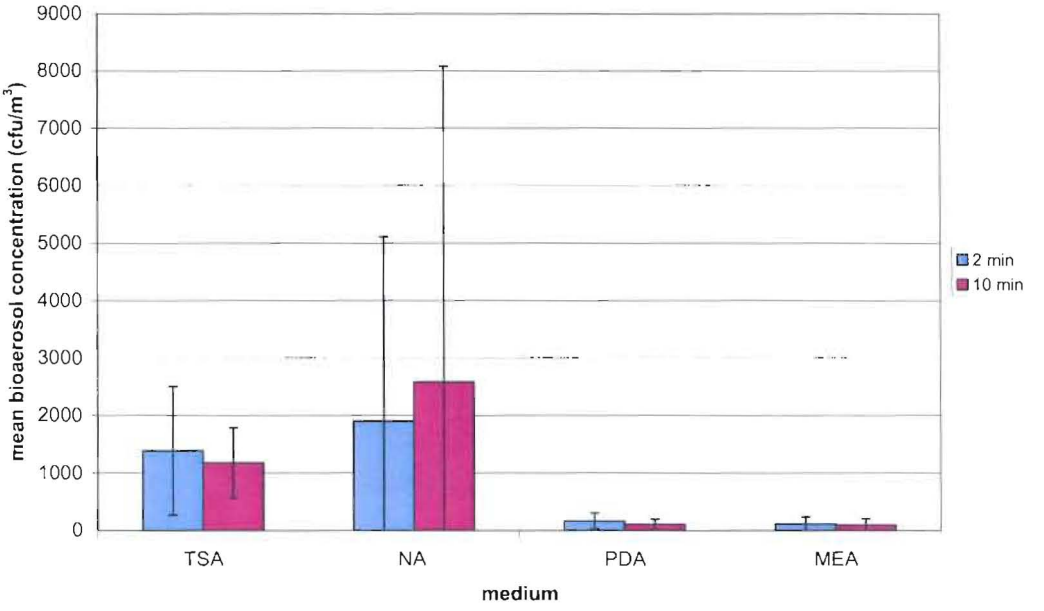


Figure 4.6: Effect on sampling time on the sampling efficiency of the Andersen sampler for four growth media (n=9, error bars show standard deviation).

The bioaerosol concentration detected using a 10 minute sample time was the same as that for 2 minutes. This is illustrated in the photograph shown in Figure 4.7. Provided the efficiency of the sampler remains constant, sampling for 10 minutes should collect five times as many microorganisms as sampling for 2 minutes. The numbers of microbial colonies found on plates that had sampled for 10 minutes were approximately five times higher than those numbers found on plates that sampled for only 2 minutes. Converting these counts to a concentration of colonies per m^3 of air sampled gave similar answers, showing that the longer sampling time of 10 minutes did not result in the collection of a higher proportion of non-viable cells.

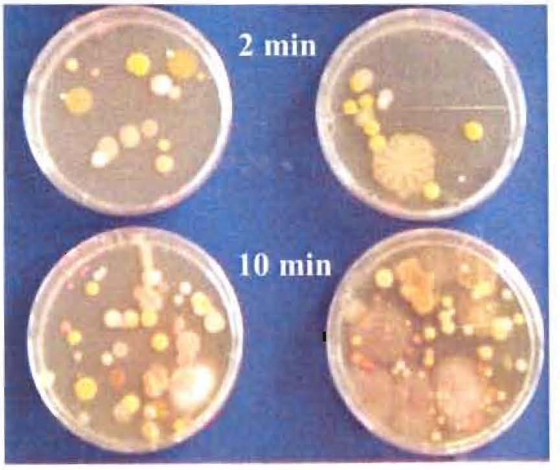


Figure 4.7: Colony numbers on 2 minute and 10 minute plates averaged over time give same viable bioaerosol concentration (Andersen sample on NA).

When examining the results for each growth medium individually, the differences in the concentrations of viable microorganisms measured over 2 and 10 minutes were not significant between the two sampling times for any of the three incubation temperatures (Figures 4.8 – 4.11).

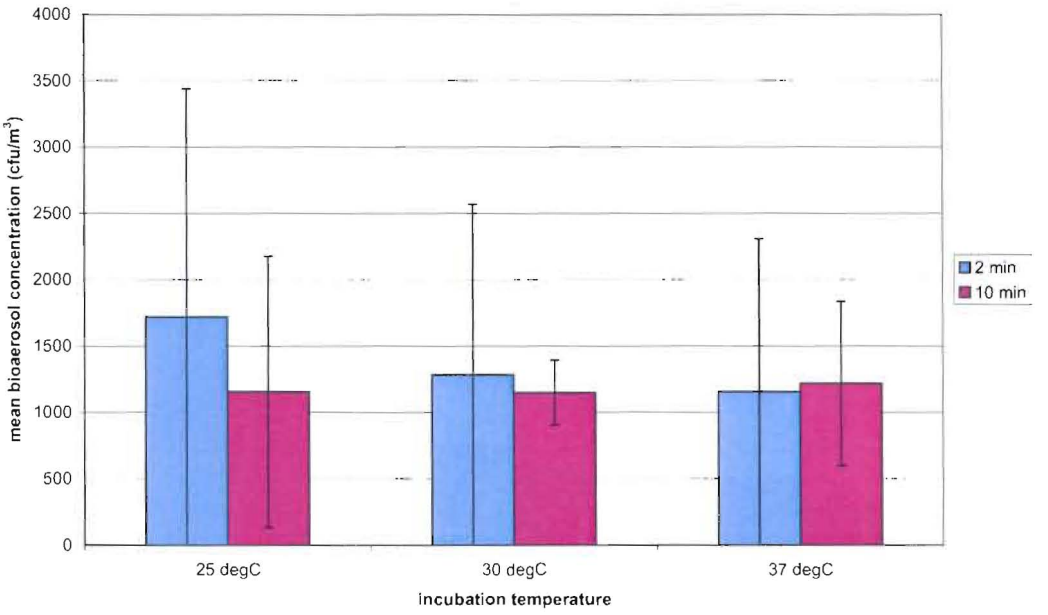


Figure 4.8: Comparison of 2 and 10 minute sampling times on TSA, across three incubation temperatures (error bars show standard deviation, n=3).

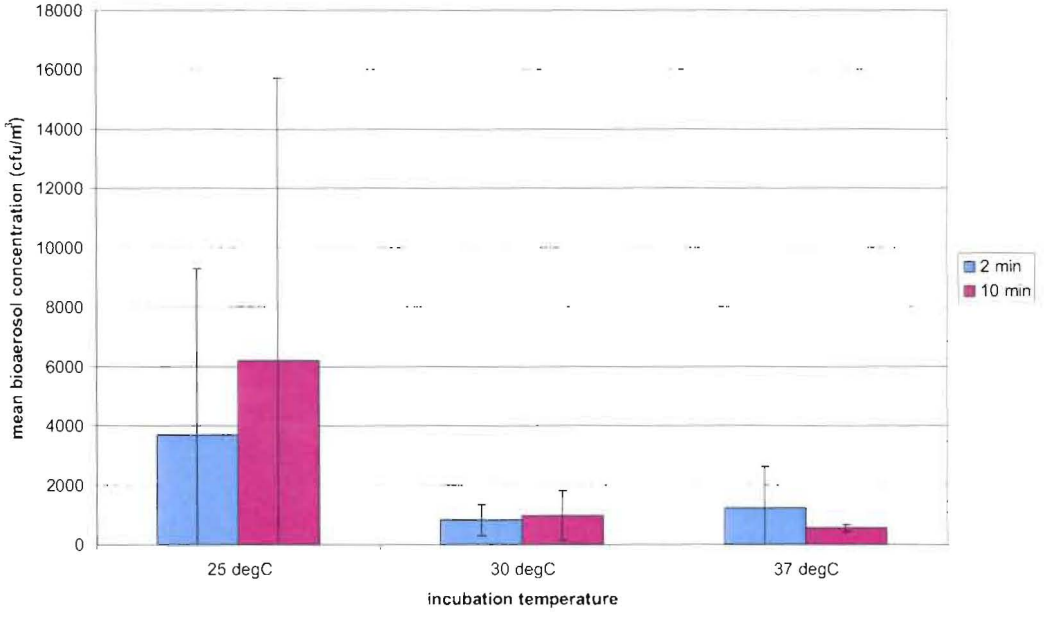


Figure 4.9: Comparison of 2 and 10 minute sampling times on NA, across three incubation temperatures (error bars show standard deviation, n=3).

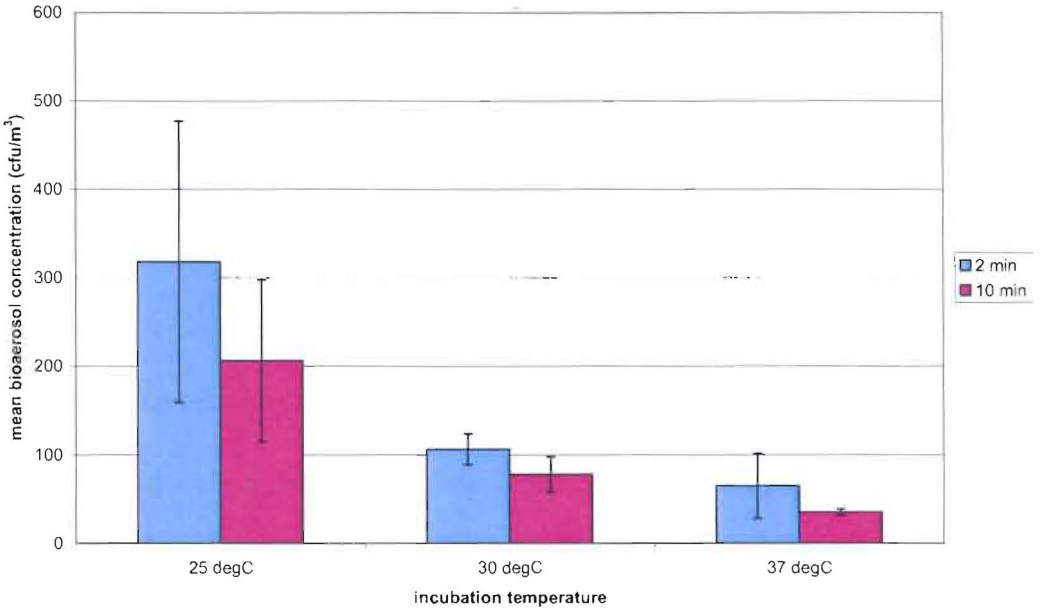


Figure 4.10: Comparison of 2 and 10 minute sampling times on PDA, across three incubation temperatures (error bars show standard deviation, n=3).

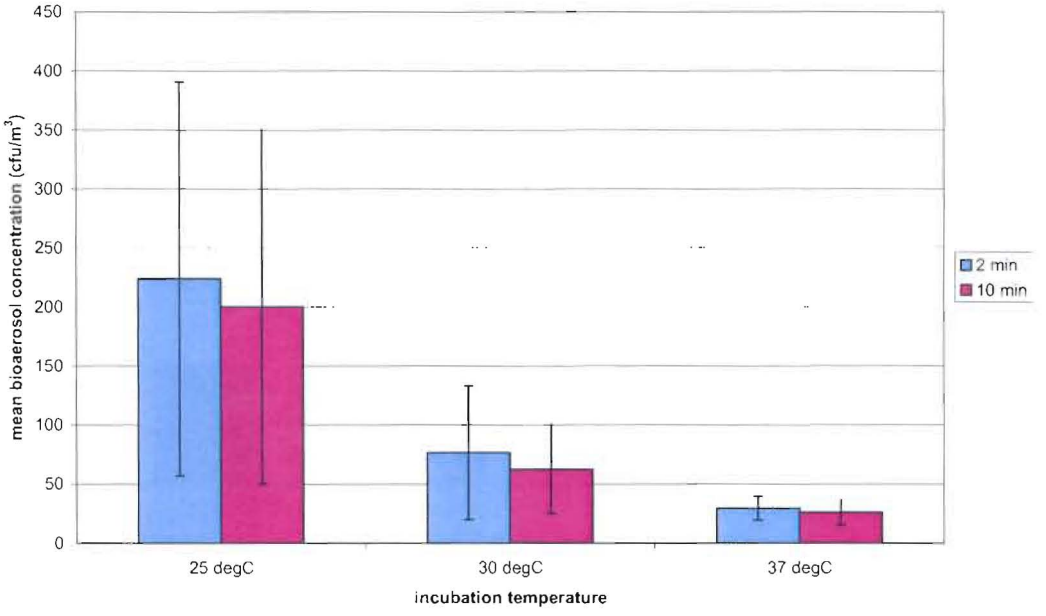


Figure 4.11: Comparison of 2 and 10 minute sampling times on MEA, across three incubation temperatures (error bars show standard deviation, n=3).

These results, however, do not agree with those quoted by Folmsbee *et al.* (2000) who suggested that longer sample times resulted in lower counts, as a result of desiccation caused by a longer sample time.

4.1.3 The effect of growth medium on the efficiency of the Andersen microbial sampler

The choice of growth medium employed for use with the Andersen sampler had a significant effect on its collection efficiency ($p < 0.05$, 2-factor ANOVA). The four media examined were: Nutrient agar (NA), Malt Extract agar (MEA), Tryptone Soy agar (TSA) and Potato Dextrose agar (PDA). It was found that, on average, for all incubation temperatures and sampling times, NA gave consistently higher colony counts than any of the three other media (Figure 4.12).

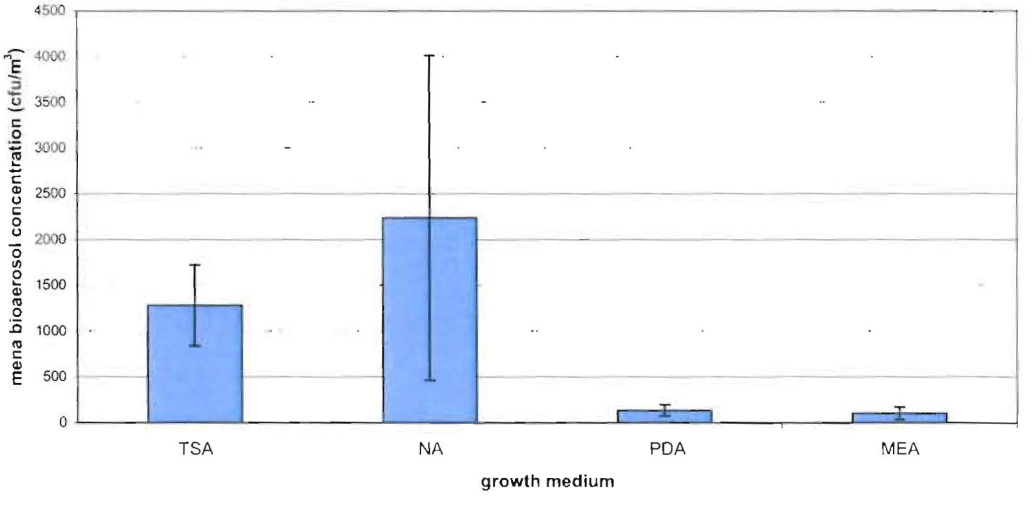


Figure 4.12: Effect of growth medium on the total efficiency of the Andersen sampler (error bars show standard deviations, n = 18).

The differences between NA and PDA, and between NA and MEA were significant ($p < 0.05$, t-test, n=18), whereas there was no significant difference between the counts obtained on NA and those on TSA (Appendix 3). For future sampling, it was concluded that both NA and MEA would be used on each occasion to obtain samples of viable bacteria and fungi, respectively.

4.1.4 The effect of incubation temperature on the efficiency of the Andersen microbial sampler

Using the combined data from both the 2 and 10 minute sample times (Appendix 4), this study has found that of the three temperatures examined, 25°C gave the highest colony counts – irrespective of which growth medium was used (Figure 4.13). On PDA the counts achieved at 25°C were significantly higher than those at either 30°C or 37°C ($p < 0.05$, t-test, n=6). On MEA, the 25°C counts were significantly higher than the 37°C counts ($p < 0.05$, t-test, n=6). Growth on TSA appears to be almost temperature-independent with very little difference between the measured bioaerosol concentrations at each temperature. For 25°C, 30°C and 37°C the measured viable bioaerosol concentrations on TSA were 1438 cfu/m^3, 1216 cfu/m^3 and 1187 cfu/m^3, respectively.

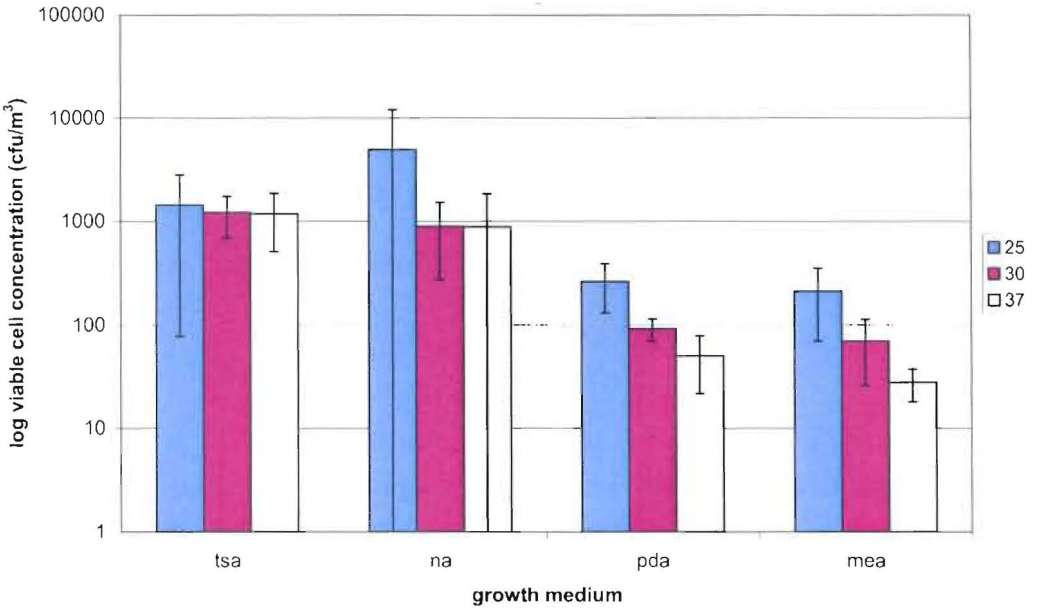


Figure 4.13: Effect of temperature on colony counts for each growth medium (error bars show standard deviations, n = 6) [NB – colony counts are expressed on a log scale to cover the large variability in results].

For the purposes of establishing an optimised method for future sampling, 30°C was selected as the 'best' temperature at which to incubate the plates, compromising the need for the highest achievable counts with the requirement of speed. In the cases of samples taken on to PDA and MEA agars especially, 30°C gave measured bioaerosol concentrations at an intermediate level between those achieved at 25°C and those at 37°C.

4.1.5 The Andersen sampler – Summary of optimum sampling conditions

From the results of the preliminary experiments previously described throughout section 4.1, it was possible to select the sampling criteria that would be best employed when using the Andersen sampler to measure indoor concentrations of bioaerosols.

A suggested standardised sampling regime for use with the 6-stage Andersen sampler is:

- Use standard disposable 90 mm plastic Petri dishes for the collection of samples.
- Run samples for 10 minutes to ensure the collection of a large enough sample.
- Take duplicate samples on to both nutrient agar and malt extract agar for successful growth of both viable bacteria and fungi, respectively.
- Incubate samples at 30°C for 72 hours.

4.2 The Omega AirTEST air sampler

The bioaerosol concentrations measured by the Omega sampler followed a similar pattern to that shown by the total airborne particulate concentrations, as measured using the LN5 laser sampler. This pattern was seen both when sampling on to NA and MEA (Figures 4.14 and 4.15).

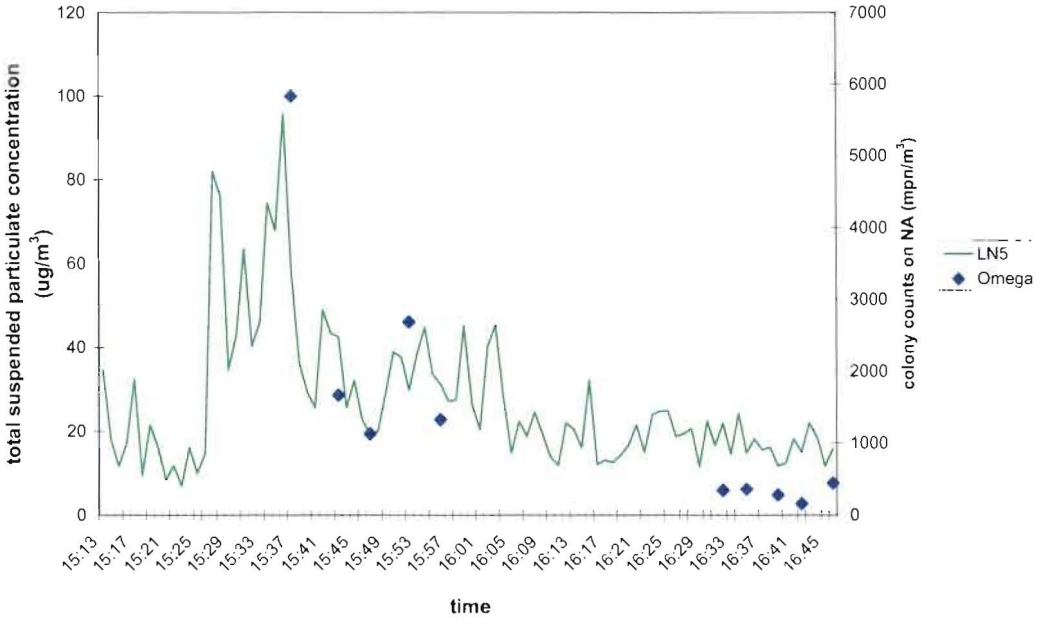


Figure 4.14: Comparison of bioaerosol concentrations on NA measured by the Omega sampler and total suspended particulate concentrations.

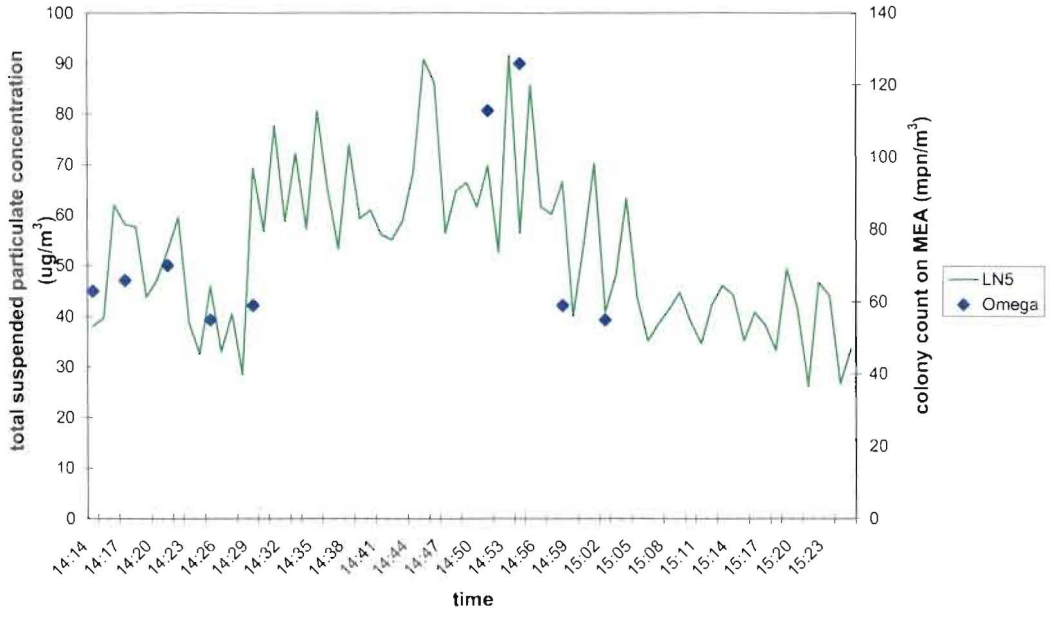


Figure 4.15: Comparison of bioaerosol concentrations on MEA measured by the Omega sampler and total suspended particulate concentrations.

On both nutrient agar and malt extract agar, the viable cell concentrations measured by the Omega sampler compared favourably with the total airborne particulate concentrations recorded by the LN5 laser monitor. As the concentration of total suspended particulates in the air increased, this was reflected by a measured increase in the numbers of viable microorganisms collected on NA and MEA.

In addition, the results obtained using the Omega sampler were highly comparable to those achieved using the Andersen sampler. Samples were taken using the Omega and Andersen samplers simultaneously, while the LN5 laser monitor was running. The viable counts from both samplers showed the same relationship, increasing with corresponding peaks in total airborne particulate concentration. The results from the Andersen sampler however, tended to be slightly higher than those obtained using the Omega sampler. An example of this relationship can be seen in Figure 4.16, where samples were taken in parallel on to nutrient agar and incubated at 30°C. Statistical analyses of these data, however, show that this difference is not significant ($p > 0.05$, Mann-Whitney). See Appendix 5.

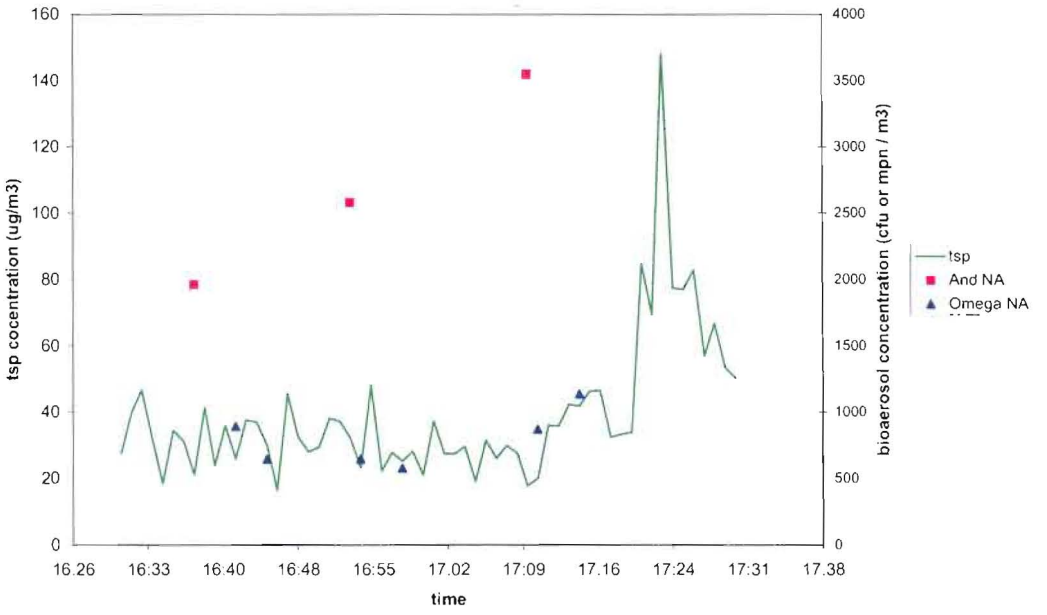


Figure 4.16: Comparison of bioaerosol concentrations measured by the Andersen and Omega samplers (NA) with total suspended particulate data.

These comparative experiments indicate that in terms of its bioaerosol collection efficiency, the Omega AIRTEST viable sampler compares favourably with the well-characterised Andersen 6-stage sampler. Additionally, it performs well in accurately reflecting the fluctuations in airborne particulate concentrations measured by the LN5 laser monitor.

4.3 The effect of wind speed and direction on Andersen and Omega sampling results

There was no measurable effect of wind strength or direction on the numbers of viable organisms collected by either the Andersen or Omega samplers, when sampling in an indoor office environment (Figures 4.17 - 4.20).

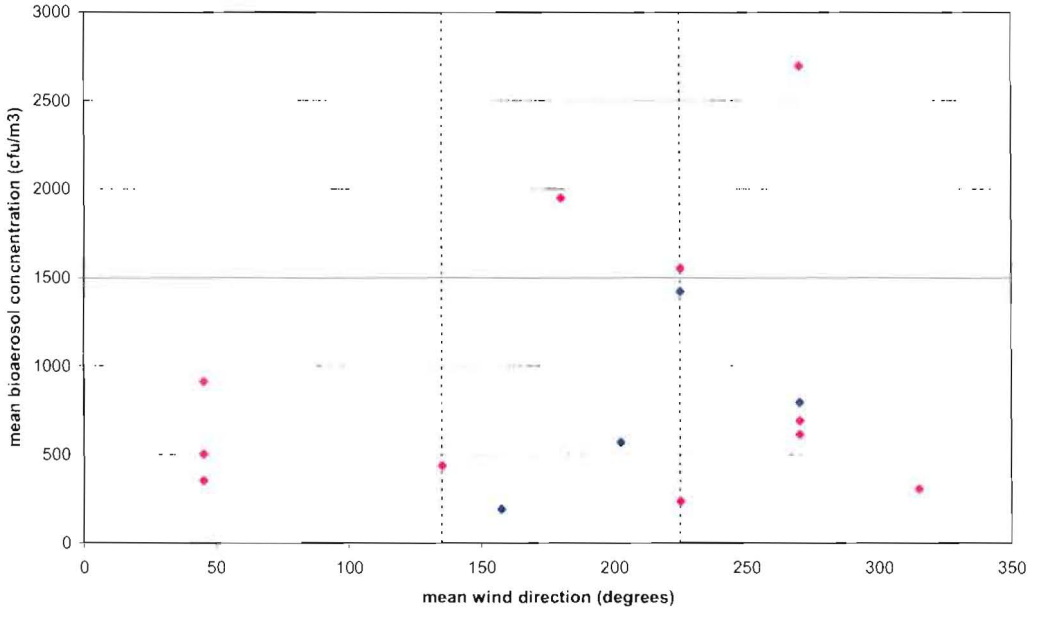


Figure 4.17: Effect of wind direction on bioaerosol collection on NA of Andersen (♦) and Omega (♦) samplers.

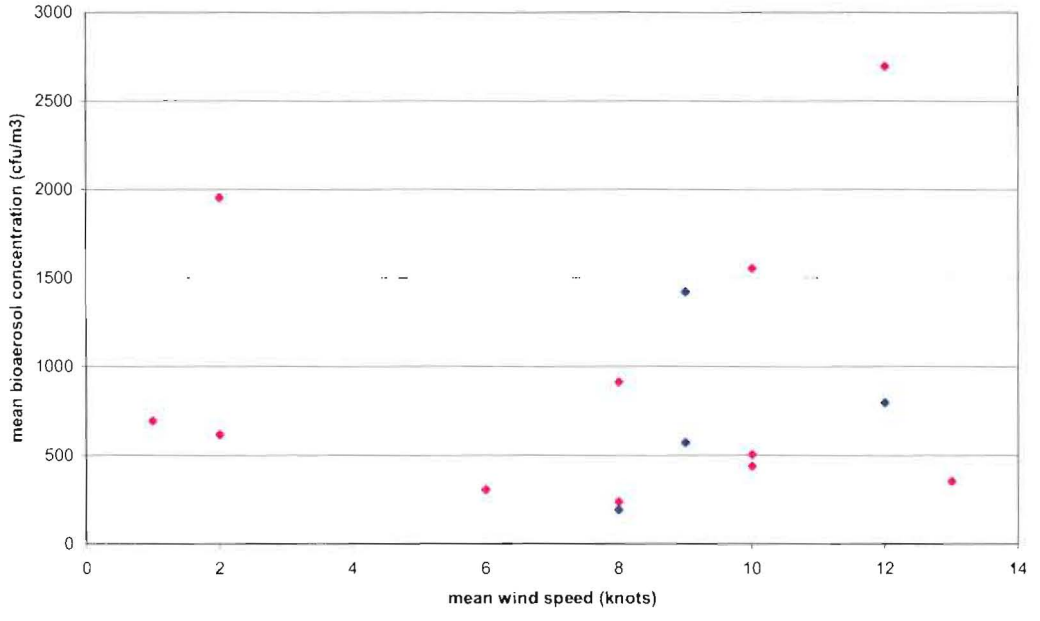


Figure 4.18: Effect of wind speed on bioaerosol collection on NA of Andersen (♦) and Omega (♦) samplers.

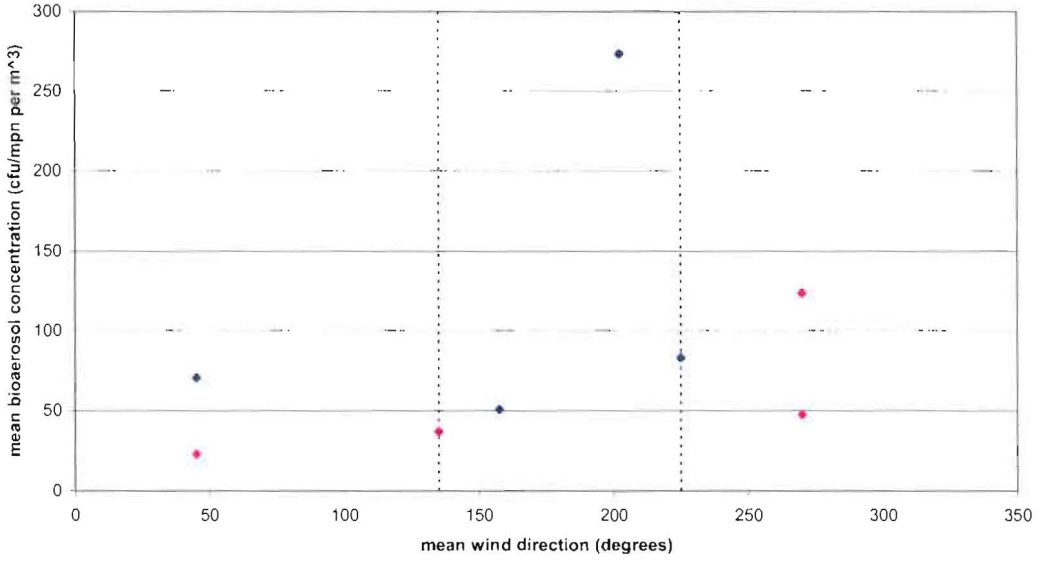


Figure 4.19: Effect of wind direction on bioaerosol collection on MEA of Andersen (♦) and Omega (♦) samplers.

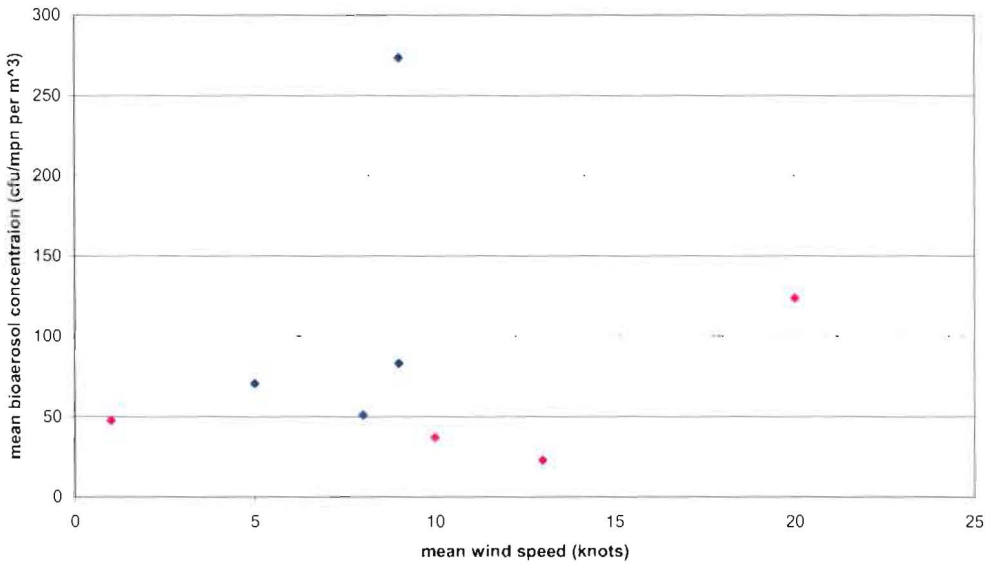


Figure 4.20: Effect of wind speed on bioaerosol collection on MEA of Andersen (♦) and Omega (♦) samplers.

There was no significant relationship between wind speed or direction for samples collected by either the Andersen sampler or Omega sampler on either nutrient agar or malt extract agar. The directional data was examined with regard to all measurements that had been taken when the mean wind direction was anywhere between south-easterly (135°) and south-westerly (225°). It was regarded that winds from this range of directions was most likely to have a direct bearing on that hitting the office windows and possibly affecting the particle influx from outside into the room. The dashed lines on the two graphs of wind direction show these degrees. It can be seen that the bioaerosol concentrations measured on the days when mean wind direction was between south-easterly and south-westerly were not significantly higher than those recorded for any other wind direction.

4.4 AGI-30 all-glass impinger

Initial results carried out in an office environment gave poor results for the AGI-30 liquid impinger. Sampling was carried out in parallel with the Andersen sampler. Where the Andersen sampler gave detectable levels of viable airborne microorganisms on both NA and MEA, the corresponding samples obtained from the liquid impinger showed no detectable microorganisms. No microbial counts were obtained from any of the dilutions, and even the undiluted sample of collection fluid gave a count of zero cfu/m^3.

Samples taken from at the surface of spore-producing colonies of both *Aspergillus* and *Penicillium* in the laboratory showed successful growth on both NA and MEA, at all dilutions of the collection fluid. The species grown on each plate also corresponded to that on the test plate. These results suggest that a more heavily contaminated environment is likely to generate more successful sampling results with the AGI-30 impinger.

The repeat samples taken in the office environment, using a longer sample time of 25 minutes, showed detectable levels of microorganisms in both the impinger samples plated on to NA and those on to MEA. The numerical results from these samples have not been included here as the viable counts achieved are not strictly important. The main result of interest is the conclusive evidence that the sample time and volume used with the impinger were suitable to isolate viable microorganisms from indoor air. Therefore, assuming that the bioaerosol concentration to be measured is high enough, the recommended conditions for use with the AGI-30 impinger are:

- A standard flow rate of 12.5 $l.min^{-1}$, as recommended by the manufacturer.
- 20 ml of ¼ strength Ringers solution, as collection fluid.
- A sample time of 25 minutes.

4.5 Filter samplers

Following the preliminary work carried out on the polycarbonate membrane filters to examine the recoverability of microbial particles from the filter surface, electron micrographs were taken of the surfaces of a) a clean, unused filter, b) a used, soiled filter and c) a used filter, following washing. These show:

a) The smooth surface of the polycarbonate filters, designed specifically for ease of removal of collected particles (Figure 4.21a).

b) The collected particles (including microorganisms) on the surface of the filter (Figure 4.21b). It is clear that the smooth surface allows particles merely to rest on the filter surface rather than becoming entangled within the filter structure. The pore size itself is also shown to be effective at trapping particles. Cells can be seen next to pores, obviously too large to pass through them and hence trapped for further analysis.

c) The surface of a polycarbonate filter post-washing (Figure 4.21c). The effectiveness of particle removal is shown by the achievability of a 'clean' surface, comparable to that before sampling was carried out.

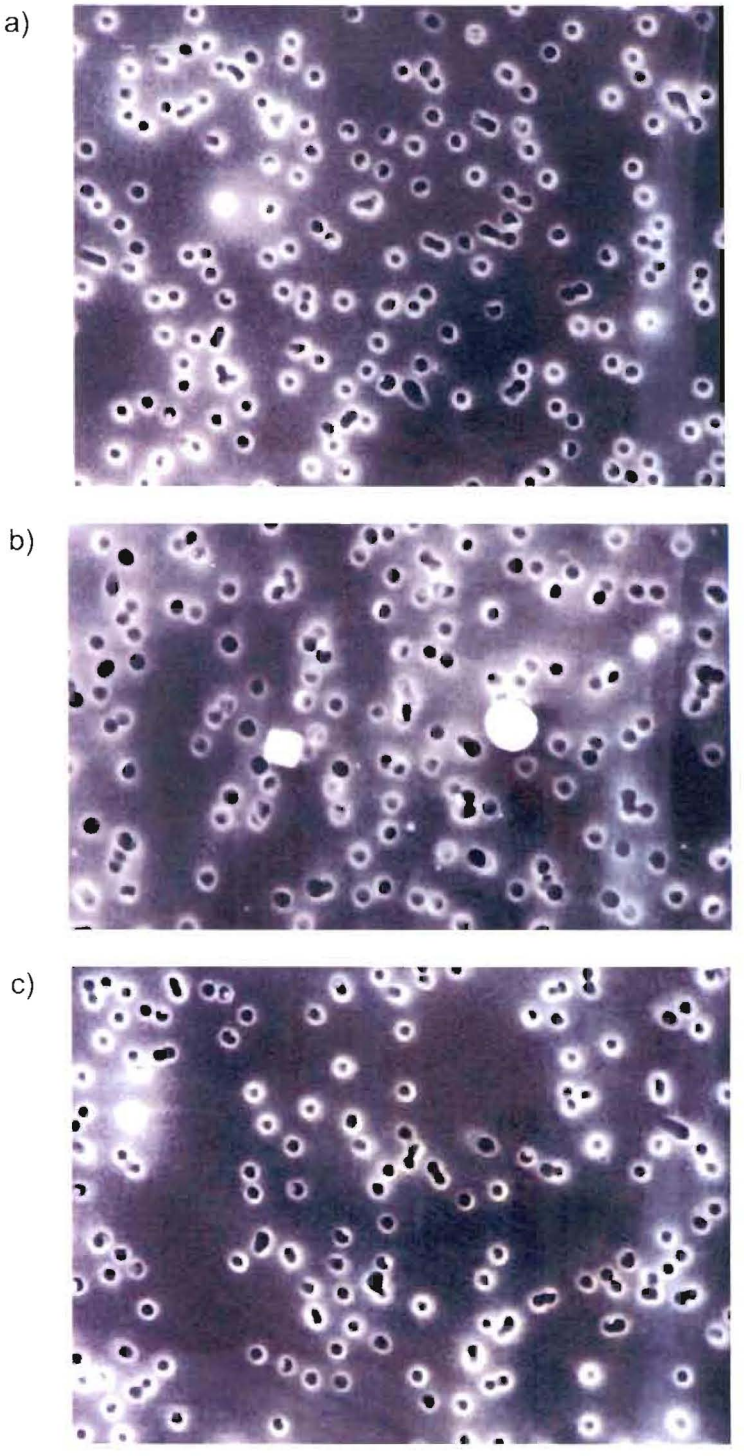


Figure 4.21: Electron micrographs of the surface of **a)** a clean polycarbonate filter **b)** a polycarbonate filter showing trapped particles **c)** a polycarbonate filter following washing (x 5000 magnification).

For comparison, an electron micrograph of the surface of a glass fibre filter that has been used for bioaerosol sampling is shown in Figure 4.22. Here, the structure of the filter itself is noticeably different to that of the polycarbonate filter. It is possible to see the tangled fibres of the filter as well as numerous particles trapped in amongst them, indicating the efficiency with which this type of filter functions. However, the unsuitability of this type of depth filter for bioaerosol sampling, where microbial particles need to be recovered from the filter once trapped, is obvious.

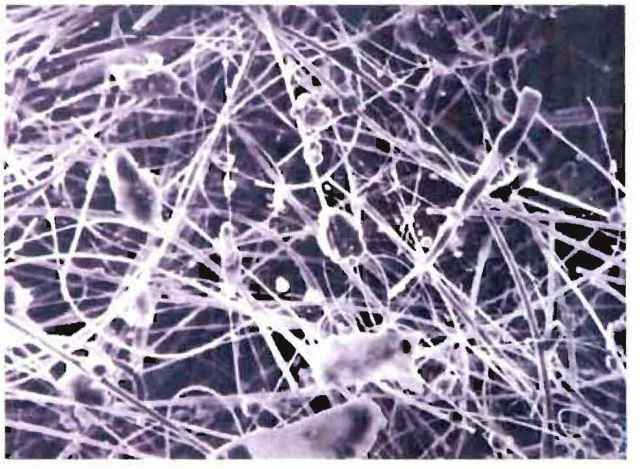


Figure 4.22: Electron micrograph of the surface of a glass fibre filter following air sampling (x 1400 magnification).

4.6 Measurement of total airborne particulate concentrations using the Negretti LN5 laser monitor

The results obtained from measurements made using the Negretti LN5 laser sampler demonstrate that airborne particulates show extreme fluctuations over time. Additionally, it can be seen that the degree of these fluctuations varies

between the different particle sizes. The greatest fluctuations over time are seen for the largest particles, while the smaller particles such as PM2.5 and PM1 have a more constant distribution.

Looking at the laser sampler data from the preliminary experiments carried out in an office environment (Figure 4.23), it can be seen that there were large variations in particulate concentration, which were clearly related to patterns of human activity.

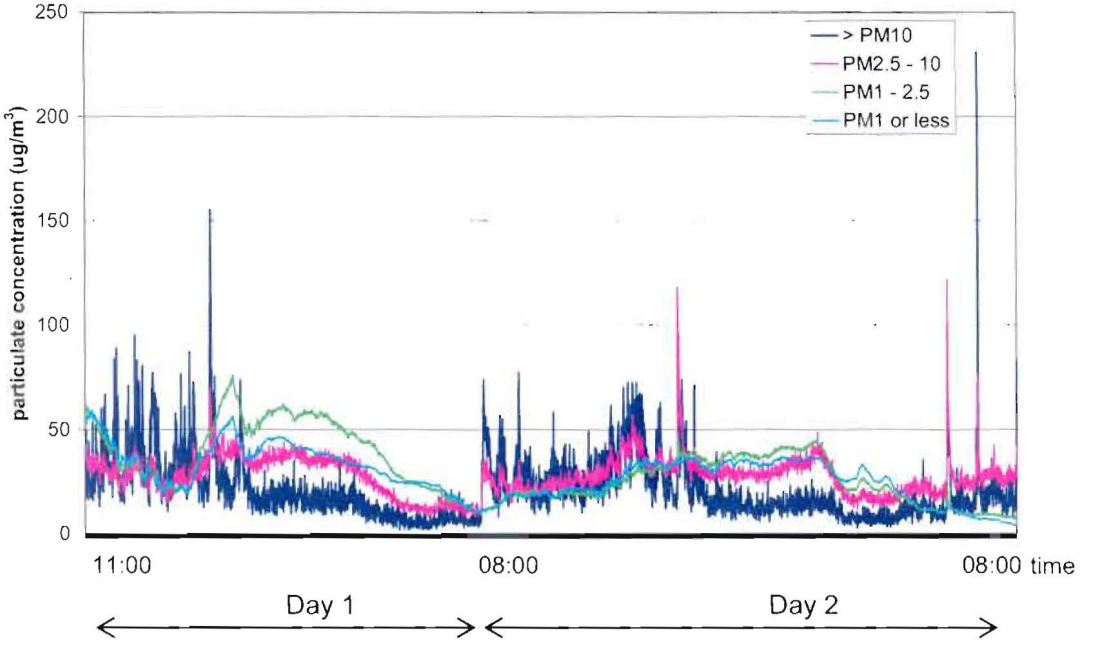


Figure 4.23: Fluctuations in total airborne particulate concentrations over time as measured by the Negretti LN5 laser sampler.

Particulate levels in the size range greater than PM10 (> PM10) decreased over night from around 155 μg m^{-3} at 18:00 h to a minimum of approximately 3 μg m^{-3}, just before human activity (in the form of cleaners and office staff) resumed at around 8 am, causing a rapid increase in the levels of airborne dust (Figure 4.23). This was emphasised with a marked decrease in particulate concentrations over the weekend period, when the office was empty (Figure 4.24). Hence, it can clearly be seen that fluctuations in particulate concentrations are related to environmental disturbances caused by human activity.

It should be noted here that the data used to plot Figures 4.23 and 4.24 was obtained during different sampling periods. That seen in Figure 4.23 was collected over a period of two weekdays and nights, while the data from Figure 4.24 came from sampling carried out over a week, including a weekend. Equivalent graphs were drawn for each sampling period and each showed the same particulate behaviour.

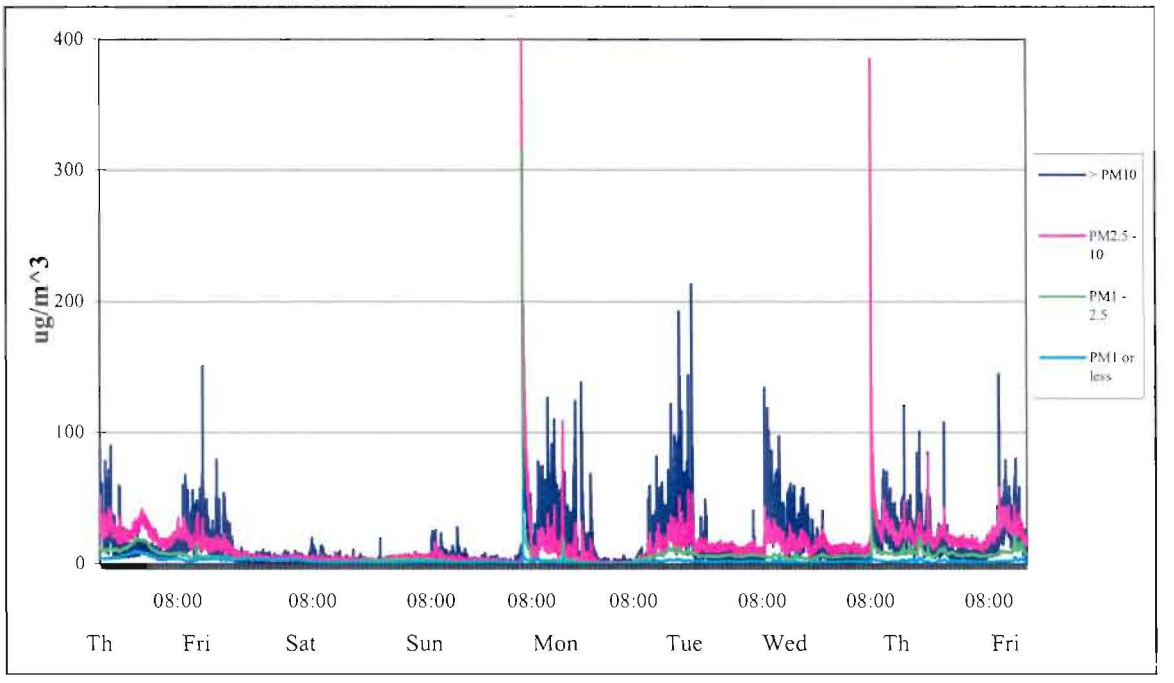


Figure 4.24: Change in airborne particle concentrations over time (as measured by the Negretti laser sampler over a weekend period).

These fluctuations should be considered not only with respect to the length of sampling time but also when deciding on the time of day at which to carry out sampling. All sampling should be carried out at a time when bioaerosol concentrations are representative of the conditions of interest. For instance, if the likely bioaerosol concentrations experienced in domestic housing are of interest, then sampling should be performed when the house is occupied, as the presence of occupants will have a large effect on the numbers of microorganisms detected.

Airborne particulate data was collected over an 8-day sampling period and the particulate concentrations at 2 pm each day were plotted (Figure 4.25). It is clear that TSP and PM 10 are subject to greater variability over time than PM 2.5 and PM 1.

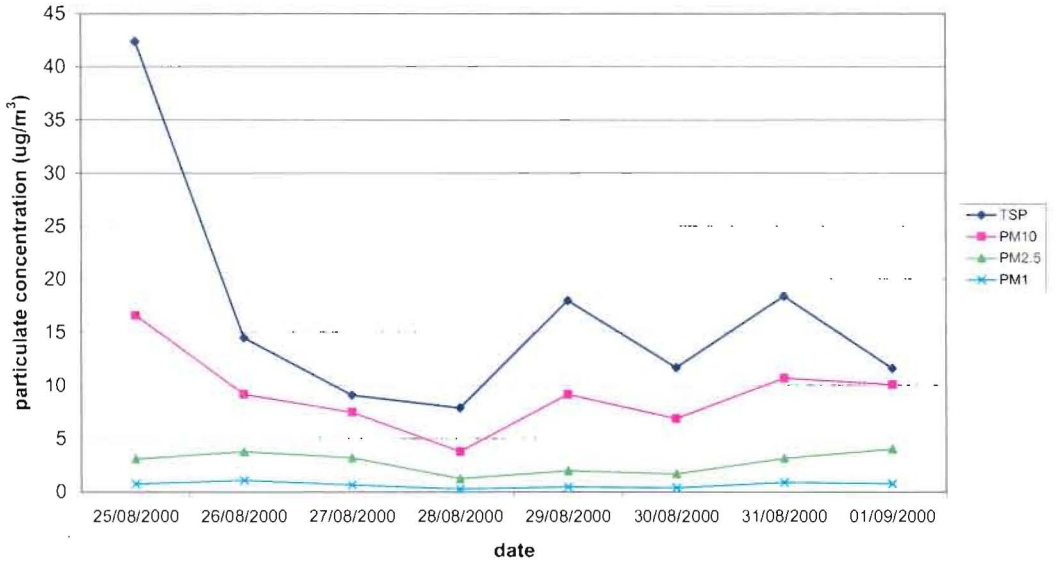


Figure 4.25: Daily fluctuations in airborne particulate concentrations in an office environment, as measured by the LN5 laser sampler.

It can be seen from Figure 4.26 that the particulate composition of indoor air varies with time. The proportion of larger particles i.e. greater than PM10 decreases at night as these heavier particles settle out in undisturbed air. The proportion of lighter particles (< PM1 – PM2.5) hence is higher at these times as they remain suspended in the air.

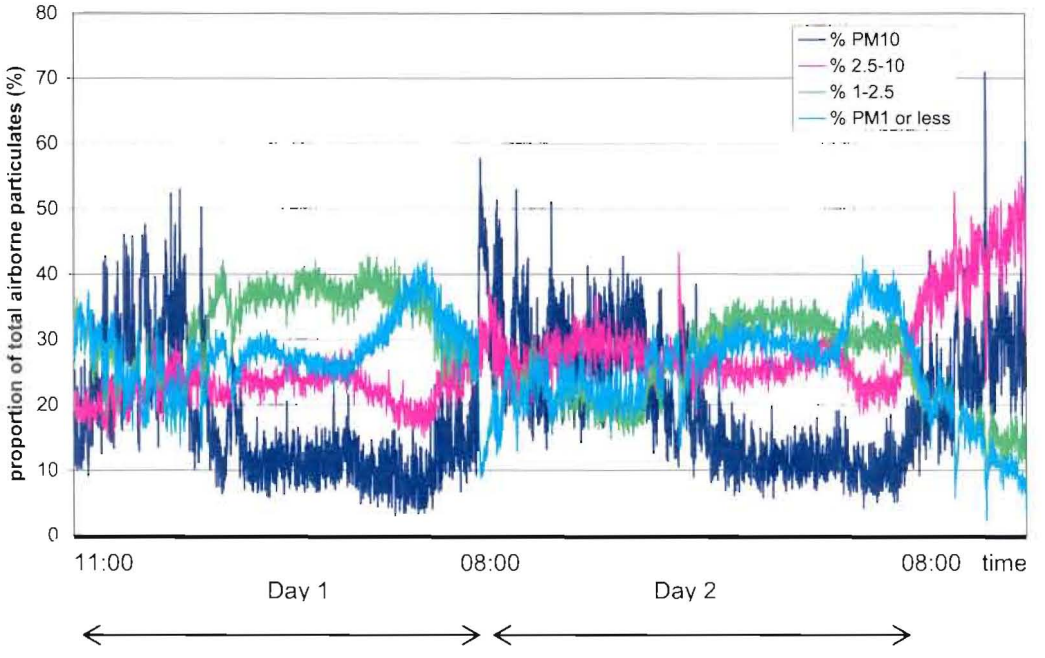


Figure 4.26: Change in airborne particulate composition over time as measured by the Negretti laser sampler.

4.6.1 Total airborne particulate concentrations and their relationship with measured viable bioaerosol concentrations

The particulate concentrations shown in Figure 4.26 were compared with the concentration of viable microorganisms collected by the Andersen sampler onto Nutrient agar, during the same sampling period (Figure 4.27).

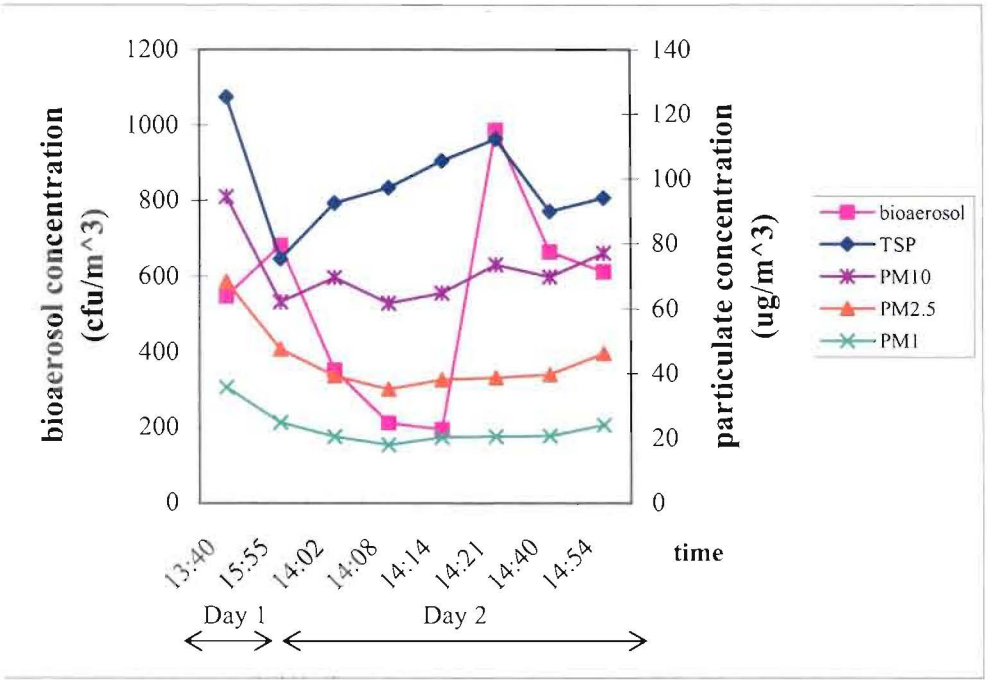


Figure 4.27: Variations over time in bioaerosol and particulate concentrations, sampling with the Andersen sampler (plastic plates, NA) and LN5 laser sampler, respectively.

It can be seen that there is a possible relationship between bioaerosol concentration and PM2.5 and PM1 concentrations, but this is not apparent for PM10 and TSP. The likely reason for this relationship may be that because Nutrient agar selects for bacteria over fungi and because bacterial cells fall into the size range of PM1 and PM2.5 particles, the fluctuations seen in the concentrations of these particle sizes are reflected in the bioaerosol concentrations collected by the Andersen sampler. However, this relationship is not clearly represented so no meaningful conclusions can reliably be drawn from these data.

Additional data, however, collected using 2 types of bioaerosol sampler to compare viable bioaerosol concentrations with particulate concentrations allowed

valid statistical analyses to be performed. For the Andersen sampler, total viable counts (totalled for all six plates) on NA were significantly correlated with mean TSP and mean PM 2.5 ($p < 0.05$, Spearman's rho) and mean PM 1 ($p < 0.01$, Spearman's rho) particle concentrations measured during the sampling period (Appendix 6). Viable counts achieved using the Omega sampler with both NA and MEA were also significantly correlated with PM 2.5 and PM 1 particle concentrations (Appendix 3). Data were not available for using the Andersen sampler with MEA. It is concluded from these analyses that total viable count is closely related to airborne concentrations of PM 2.5 and PM 1.

Further analyses of these data were performed to examine the relationship between total viable counts on each of the six stages of the Andersen sampler and mean particulate concentrations. It was hypothesised that there would be a relationship between the total viable counts on each plate and the concentration of the particulate size that most closely corresponded to the size fraction collected by that stage of the Andersen (Table 4.1).

Table 4.1: LN5 particulate size fractions and corresponding Andersen stage number

Stage	Particle size cut-off (μm)	Equivalent particulate size fraction
1	7 and above	TSP & PM 10
2	4.7 – 7	
3	3.3 – 4.7	
4	2.1 – 3.3	PM 2.5
5	1.1 – 2.1	
6	0.65 – 1.1	PM 1

Spearman's rank correlation was used to examine the relationships between measured bioaerosol and particulate concentrations. It would be reasonable to assume that there may be a correlation between both TSP and PM 10 concentrations and the total viable counts collected on each of the Andersen's six stages.

The results from the statistical analyses can be seen in Appendix 7. There was a significant positive correlation between the average TSP concentration over the period of sampling and the viable bioaerosol concentrations measured on each of stages 3, 4 and 5 ($p < 0.05$, $n=37$). Graphically, there was also evidence of similar correlations with the counts on plates 1, 2 and 6. These results, however, were not found to be statistically significant. Unexpectedly, there were significant positive correlations between the average PM 1 concentration and the viable bioaerosol concentrations measured on each of stages 1 – 6. For stages 1, 3, 4 and 5 this

relationship was highly significant ($p < 0.01$, n=37). P-values of < 0.05 were achieved for stages 2 and 6. These results may warrant further investigation.

These data also showed the relationship between each particle size fraction. Highly significant positive correlations were found between average TSP and average PM 10 concentrations ($p < 0.01$, Spearman's rho) and between average PM 2.5 and PM 1 concentrations ($p < 0.01$, Spearman's rho). There was no evidence of a significant relationship between either of the two larger size fractions (TSP and PM 10) and the smallest fractions (PM 2.5 and PM 1).

4.7 Surface sampling using Dustbuster® vacuum cleaner

The experiments carried out to take surface samples of floor dust were successful with respect to effectively collecting microbially-laden dust that could be analysed for numbers and types of species using culture-based methods. The actual bioaerosol concentrations measured during these preliminary investigations are not mentioned here but the most commonly found bacterial and fungal species are described. Floor dust samples from an office environment contained mainly bacterial species as well as a small proportion of fungi. Yeasts were also found in some samples. An example of a typical culture plate from a vacuumed sample of floor dust is shown in Figure 4.28.

Figure 4.28: Typical bacterial and fungal colonies isolated from carpet dust.

The main bacterial species isolated from settled carpet dust were *Micrococcus* and *Staphylococcus*. Isolated fungi tended to be *Penicillium*, *Cladosporium* or sterile mycelia.

Further results on the effectiveness of collection and the species identified from samples taken in indoor occupational and residential environments can be found in Sections 4.11 and 4.16.3.

4.8 Identification of most common airborne isolates

From the preliminary air sampling carried out in this study, a wide variety of microorganisms were identified as having been most commonly isolated from indoor air (Figure 4.29). These identifications (Tables 4.2 and 4.3) were used for classifying all subsequent samples taken in this study. Microbial species were identified in this way to provide a means of comparison between samples rather than an absolute qualification of the precise species composition of each sample.

The distribution of commonly identifiable species was examined across each media type, in relation to incubation temperature, sampling time and sampler stage. It was hoped to be able to identify particular species found only under certain conditions and to be able to use these as 'indicator species'. This would be of particular use in determining which sampling regime is most likely to detect or miss the commonest airborne species in a particular indoor environment.

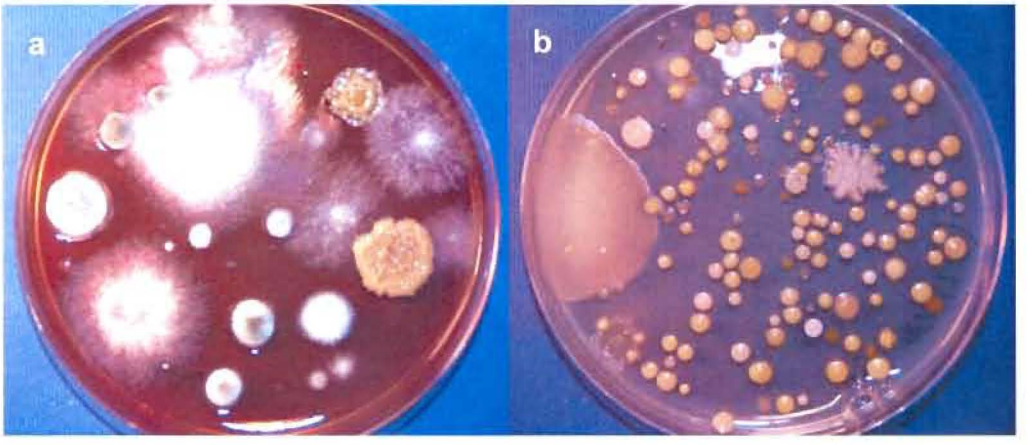


Figure 4.29: Sample plates from Andersen sampling in an indoor office environment, showing the range of microorganisms commonly isolated on a) Malt Extract agar and b) Nutrient agar.

Using the API identification system, the most commonly isolated bacterial species from all the samples were identified. The API kits indicated that these ranged from *Bacillus subtilis* and *Micrococcus luteus*, to *Staphylococcus spp* (Table 4.2).

Table 4.2: Likely identifications of several of the most common bacterial species isolated from indoor air using the Andersen sampler

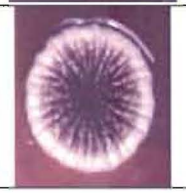
Colony	Colony morphology	Gram stain & cell type	Likely identification
	White, shiny, round.	Very small, G +ve cocci	*Staphylococcus spp*
	Creamy, dry, flat.	G +ve cocci	*Micrococcus spp*
	Yellow, smooth, shiny.	G +ve cocci	*Micrococcus luteus*
	Bright yellow, dry rough texture.	Very small, G –ve cocci	No identification *
	Beige, shiny, flat.	G +ve cocci	*Micrococcus spp*
	Orange, smooth, shiny.	G +ve rod	*Bacillus subtilis*

* obtained biochemical identification but could not obtain species identification – may not be within range covered by API kits

The API system, however, is not suitable for the identification of fungi. Fungal species were identified using traditional microbiology and microscopy. As with the bacterial species, several fungi were easily identifiable as being commonly isolated (Table 4.3). These included *Penicillium spp* and *Cladosporium spp*, both

commonly isolated from indoor air sampling (Hyvärinen *et al.*, 1993 & 2001; Madelin, 1994; Dotterud *et al.*, 1995; Hunter *et al.*, 1998).

Table 4.3: Likely identifications of several of the most common fungal species isolated from indoor air using the Andersen sampler.

Colony	Colony morphology	Likely identification
	Powdery dark green surface, thin white edge, black underneath.	*Cladosporium spp.*
	Fluffy grey-white, large black-brown conidiophores.	*Mucor spp.*
	Powdery green surface, white edge, pale underneath.	*Penicillium spp.*
	Dense white fluffy surface, distinct mass of black conidiophores.	*Aspergillus spp.*

4.9 Acridine orange staining

Preliminary staining experiments carried out on air samples obtained from an indoor office allowed the identification and enumeration of viable and non-viable bacterial and fungal cells, when viewed using epifluorescence microscopy. Problems were encountered during the initial stages of this procedure and many slides were stained only faintly, with very little fluorescence visible under the UV microscope.

Low fungal spore counts were recorded, both viable and non-viable, compared to those results obtained using culture based methods, including the Andersen and Omega samplers. Tests carried out using the spores from known cultures of *Aspergillus* and *Penicillium* species showed that increased fluorescence was achieved when the staining time was increased from 5 minutes to 1 hour. After a staining time of 5 minutes no spores, either viable or non-viable, were visible under UV. When the stain incubation time was increased to 1 hour, stained fungal cells were seen to fluoresce successfully. It should be noted that spores from *Aspergillus niger* were more resistant to staining than those of *Penicillium* species.

Further air samples were then taken from the office air, as previously, and the samples treated according to the modified staining protocol described above. Staining results were significantly improved with bacterial and fungal cells clearly visible in the samples (Figure 4.30).

Figure 4.30: Acridine orange stained air sample (stain incubation time = 1 hour), observed using epifluorescence microscopy (x 400 magnification).

4.10 Effect of human activity on bioaerosol concentrations in an office

As seen from the results described in Section 4.6, the environmental disturbance caused by human activity in an indoor room has a dramatic and measurable effect on the concentrations of airborne particulate matter in that room.

Further sampling experiments were carried out using the Andersen and Omega AIRTEST samplers to investigate the pattern of fluctuations in bioaerosol concentrations in an occupied office. Additionally, these patterns were compared directly against particulate data collected simultaneously using the LN5 laser sampler.

Each set of results was plotted to compare bioaerosol concentrations on NA and MEA, as measured by the Andersen and Omega samplers, against particulate concentrations. Each set of data showed the same clear relationship between

bioaerosol and particulate concentrations. In addition, each observed increase coincided with the incidence of human activity and environmental disturbance in the sample room. An example of the type of data collected is shown in Figure 4.31. It shows the results of synchronized samples taken in an office from early morning, covering the time of influx of people to the room and the normal activities carried out in that room. (See also Figure 4.16, which shows Andersen and Omega sampling results in conjunction with particulate data).

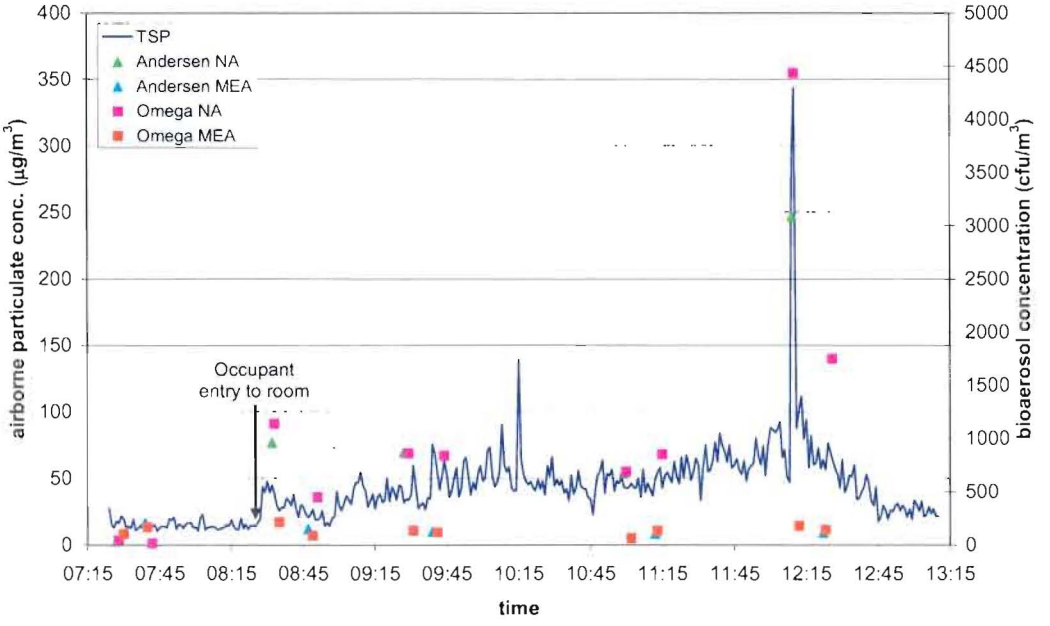


Figure 4.31: The pattern of viable bioaerosol concentrations on NA and MEA, as measured by the Andersen and Omega samplers, and total airborne particulate concentrations, showing the influence of human activity.

The relationship that can be seen between TSP and viable bioaerosol concentrations on both NA and MEA is clear for samples taken using the Andersen sampler as well as the Omega AIRTEST. However, for both the

Andersen and Omega samplers, total viable bioaerosol concentrations on NA reflect the fluctuations in particulate concentration better than those on MEA.

Initial background levels of both particulates and bioaerosols were very low from 07:20 h while the office was empty until it became occupied at approximately 08:30 h. Visible peaks were then observed in both sets of data, easily attributable to the influx of people to the room as the working day began. The mean TSP concentration over the initial undisturbed period was 15.0 $\mu g.m^{-3}$ before increasing to around 50.0 $\mu g.m^{-3}$ at 08:30 h when the first people started to arrive. Initial viable bioaerosol concentrations on NA for the Andersen and Omega samplers were 32 $cfu.m^{-3}$ and 43 $mpn.m^{-3}$, respectively. With the first incidence of human activity these measured levels increased to 961 $cfu.m^{-3}$ and 1138 $mpn.m^{-3}$, respectively. Throughout the morning, normal changes in room occupation and occupant activity were observed and the measured bioaerosol concentrations and particulate levels varied in accordance with this. The largest peak in both airborne particulates and bioaerosols was seen at around 12:15 h, at the start of the lunch hour. At this point, counts on NA increased to 3088 $cfu.m^{-3}$ and 4435 $mpn.m^{-3}$ for the Andersen and Omega samplers, respectively. This was also reflected by a TSP concentration of 343.7 $\mu g.m^{-3}$ at this same time point.

In contrast to the Andersen results on NA, the bioaerosol concentrations measured on MEA were highest at the start of the sampling period and continued to decrease steadily over time until a small increase at 12:22 h, coinciding with the major peak in particulate concentrations. Total concentrations on MEA fell from

205 cfu.m^{-3} at 07:39 h to 106 cfu.m^{-3} at 11:12 h before rising to the final measured concentration of 113 cfu.m^{-3}.

It was observed that counts achieved on NA using the Omega sampler were frequently higher than those from using the Andersen sampler. However, further analysis revealed that the mean total counts on NA for the Andersen and Omega samplers were 1127 cfu.m^{-3} and 1106 mpn.m^{-3}, respectively. This difference was not significant ($p > 0.05$, Mann-Whitney). See Appendix 8.

Mean total counts achieved on MEA were also compared for the two samplers (Appendix 8). These were 140 cfu.m^{-3} and 134 mpn.m^{-3} for the Andersen sampler and Omega AIRTEST, respectively. There was no significant difference between these results ($p > 0.05$, Mann-Whitney), again confirming the comparability of the sampling results generated by the Andersen and Omega samplers.

It can be concluded that there is a measurable effect of human activity on the viable bioaerosol concentrations in an office environment

4.11 Investigating the likely source of indoor bioaerosols in an office environment

The results from the vacuumed dust samples from the floor and occupants of several office environments can be used to investigate the possibility of the effect of the 'personal cloud', a phenomenon that is based upon the presence of

microbial particles in the air that are associated with people i.e. particles that have been 'released' from them.

Results from the surface sampling of carpeted office floors and the head and shoulders of the room occupants were analysed using both culture-based and fluorescent staining and microscopy techniques.

Looking at the results from the culture-based method, higher total viable concentrations were obtained from growth on NA, than MEA, for both floor and people samples (Figure 4.32). This difference between media was statistically significant ($p < 0.05$, Mann-Whitney) for people samples (Appendix 9). However, for floor samples, the counts on NA, although higher, were not significantly different ($p > 0.05$, Mann-Whitney) from those found on MEA. A reason for this is likely to be the large variability in results between individual samples. For example, the total viable concentrations on MEA ranged from 0 to 8111 $cfu.m^{-3}$ for both floor and personal samples. Similar variability was seen with the samples plated on to NA.

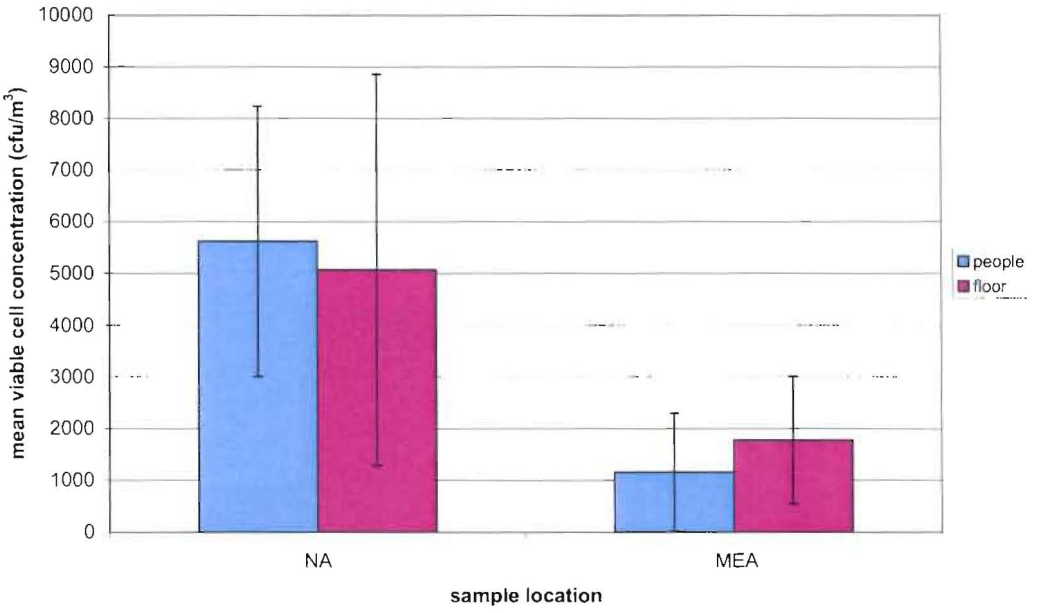


Figure 4.32: Mean concentration of total viable colonies grown on NA and MEA for vacuumed dust samples from floors and people (error bars show standard deviations, n = 12).

On both NA and MEA, the overall mean concentration of fungal colonies was slightly higher for floor samples than for samples taken from people (Figure 4.33). The mean fungal concentrations for personal and floor samples on NA were 14 and 69 fungal cfu.m^{-3}, respectively. On MEA, the mean measured concentrations were 46 and 88 fungal cfu.m^{-3}, respectively. Similarly to the results for the total viable counts, these differences were not significant ($p > 0.05$, Mann-Whitney).

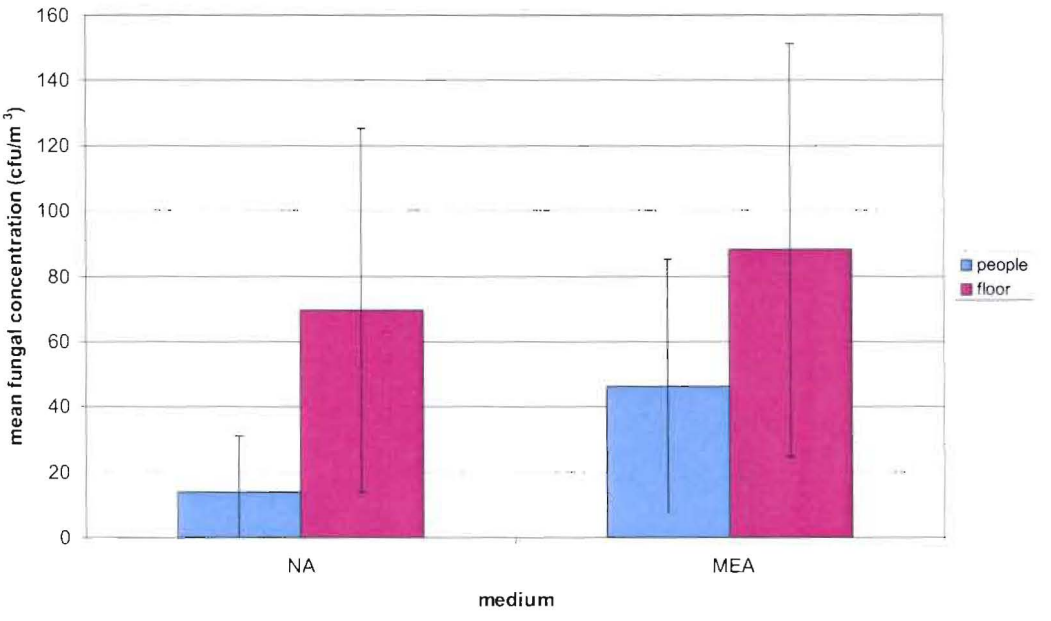


Figure 4.33: Mean concentration of fungal colonies in vacuumed dust from floor and people samples for NA and MEA (error bars show standard deviations, n = 12).

In addition to examining the actual numbers of fungal colonies for each sample, the proportion of the total number of viable colonies that was made up by fungi was also looked at. On both NA and MEA, the proportion of total colonies that were fungal was greater for samples taken from the floor. This was true for both individual samples (looking at paired person/floor results) as well as for overall results. Figure 4.34 shows the combined results for all floor and people samples on NA and MEA. On NA, 0.2% of the total number of colonies isolated from the occupant samples were fungal, compared to 1.4% of floor samples. On MEA, as expected, the proportion of fungal colonies isolated was higher for both people and floor samples with results of 4.0% and 4.9%, respectively. Statistical analyses (Appendix 9), however, showed that the higher proportion of fungal

colonies found in floor dust samples than personal samples was not significant ($p > 0.05$, Mann-Whitney).

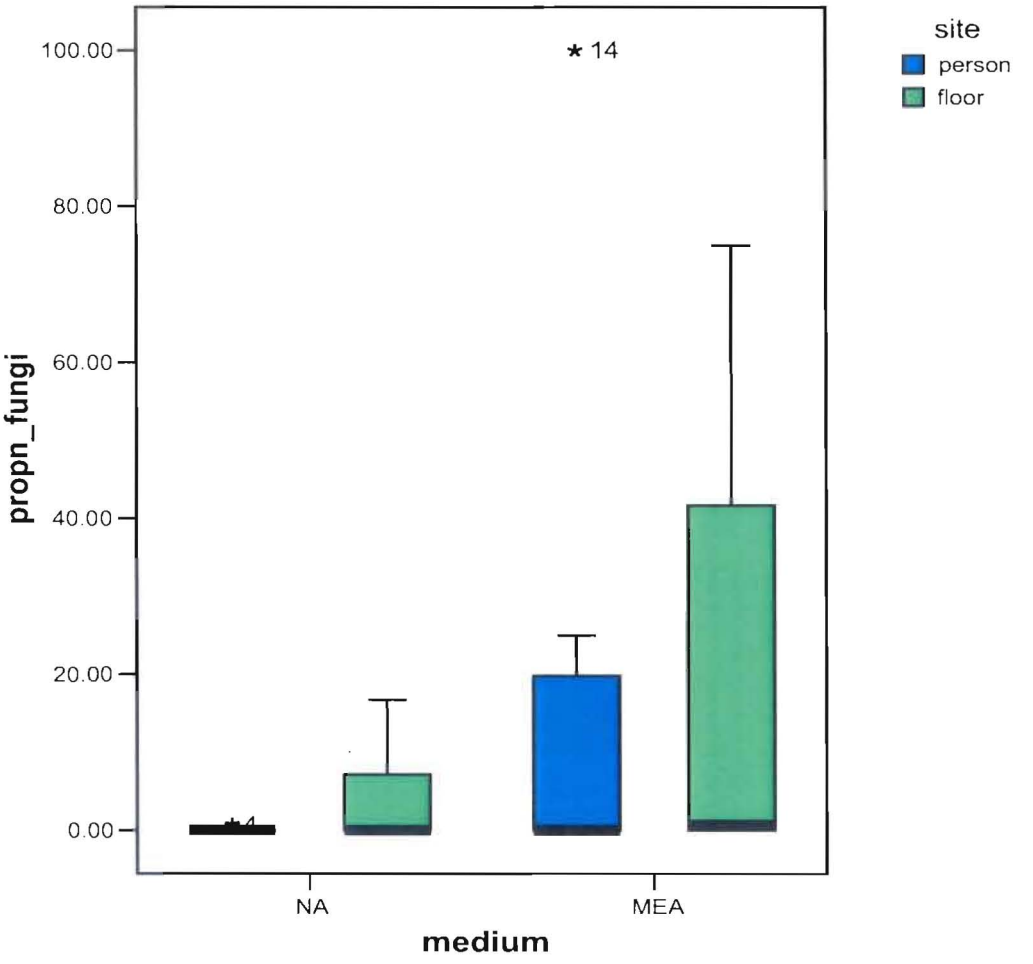


Figure 4.34: Boxplot to show the proportion of the total number of viable colonies isolated in each sample on both NA and MEA that were fungal (n=12).

It was concluded that there may be a higher proportion of fungi in samples of dust isolated from floor surfaces of indoor offices than in dust samples from the people occupying those rooms. However, very few fungal cells were actually found in the dust samples from either location

The results from the DEFT analysis of these same samples gave similar results from those obtained following culture of the filter wash fluid. Figure 4.35 shows that there were significantly higher concentrations of total bacteria, viable and non-viable bacteria in the floor dust samples than in those samples taken from people ($p < 0.05$, Mann-Whitney). See Appendix 9.

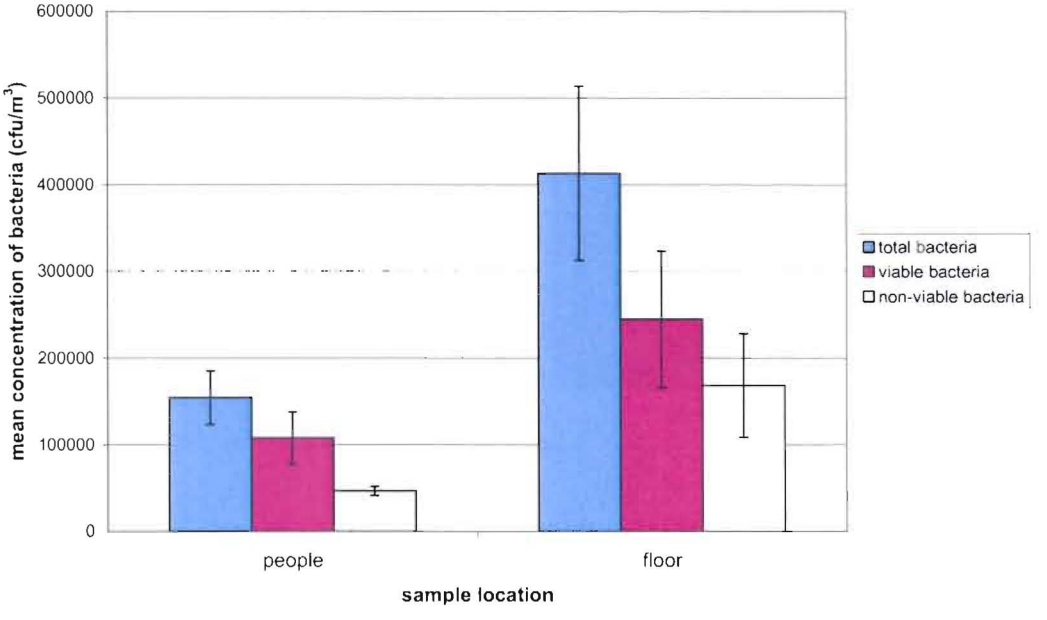


Figure 4.35: Comparison of concentrations of total, viable and non-viable bacteria in surface dust samples from room floors and occupants, as measured by acridine orange DEFT (error bars show standard deviation, n=4)

Concentrations of fungi, as calculated from the DEFT staining appear to show the same pattern (Figure 4.36). Visual inspection of the graph of results initially suggests that the levels of total, viable and non-viable fungi were higher in the dust samples taken from the floor. However, these higher results for the floor samples are not significantly different from the personal samples ($p > 0.05$, Mann-

Whitney), as indicated by the error bars of standard deviations (appendix 9). As seen previously, in Figures 4.26 and 4.27, there was a high degree of variability between each sample, affecting the significance of any statistical tests.

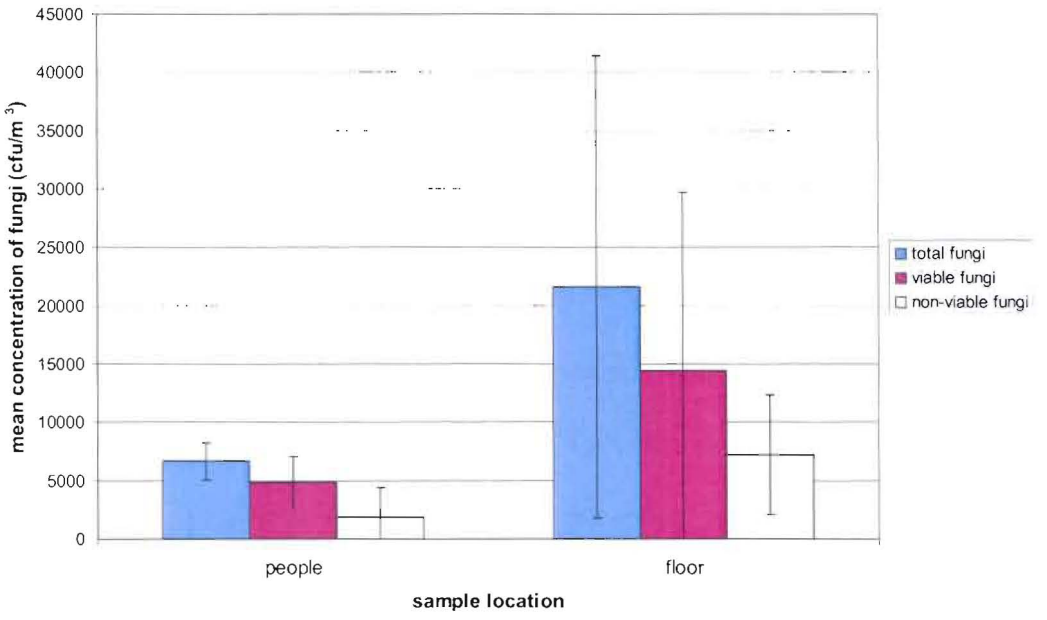


Figure 4.36: Comparison of concentrations of total, viable and non-viable fungi in surface dust samples from room floors and occupants, as measured by acridine orange DEFT (error bars show standard deviation, n=4)

On the basis of the results obtained from culturing and staining the microbiological component of the dust samples it would appear that there is a higher concentration of microorganisms, both fungal and bacterial, in floor dust than that sampled from human subjects.

Further testing was carried out using API biochemical test kits to identify the most common bacterial species isolated from each sample type. Dust samples taken

from room occupants most commonly contained *Micrococcus* spp (frequently *M. luteus*), *Staphylococcus* spp (including *S. aureus*, *S. epidermidis* and *S. capitis*, all associated with human microflora) and *Bacillus* spp. Floor dust samples also contained large proportions of *Micrococcus* and *Staphylococcus* species.

Microscopic examination of the dust samples after staining gave a very different picture of the fungal composition of each sample to that achieved using culturing. Mean viable fungi concentrations of 30 cfu/m^3 and 79 cfu/m^3 were recorded for personal and floor dust samples, respectively, when grown on agar. In contrast, the acridine orange staining method gave viable fungi concentrations of 4802 cfu/m^3 from people and 14407 cfu/m^3 for floor samples. Both results provide evidence that carpeted floor and room occupants are significant potential reservoirs for bioaerosols.

From these results it appears that the more vigorous the disturbance activity, the higher the bioaerosol concentration measured by the Andersen sampler. The mean bioaerosol concentrations measured during non-disturbance, bed-making and vacuuming were 438, 3721 and 35583 cfu/m^3, respectively, with vacuuming resulting in the greatest level of exposure to bioaerosols.

On examining Figure 4.37, however, it appears that there may be an effect of time on the measured bioaerosol concentrations. It could be suggested that bioaerosol concentrations are merely increasing with each disturbance activity due to the fact that between each sample period there has been insufficient time for the airborne particulates in the room to settle out again to their base level. As time progresses, increased activity may cause greater levels of particulate material to become and remain airborne and therefore, the later the sample is taken, the higher the measured bioaerosol concentration.

Data collected on a different sample day however, (Figure 4.38) disagrees with this theory.

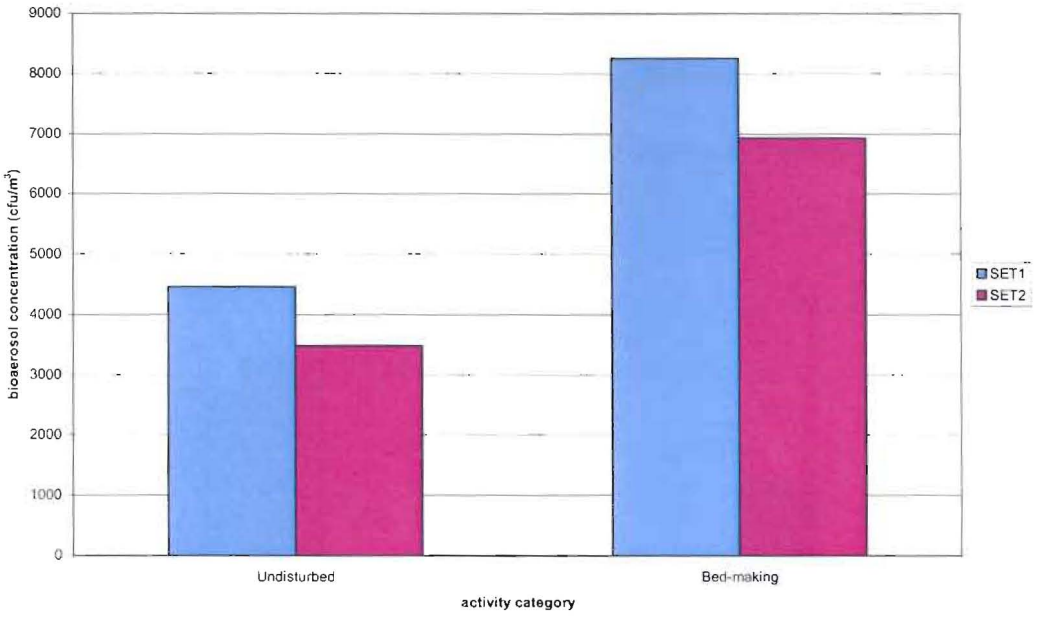


Figure 4.38: Bioaerosol concentrations from Andersen sampling (group 2) in a damp bedroom during different disturbance activities – set 1 and set 2 are repeats of the same activity category (n=1).

The results of the sampling shown in Figure 4.38 was carried out in the same way as that in Figure 4.37, but in this case there does not seem to be an effect of time on the sampling results. In addition, the results shown in Figure 4.38 agree with those in Figure 4.37 and the hypothesis that the concentration of bioaerosols detected is dependent upon the different human activities being carried out during the sampling period.

In order for any meaningful conclusions to be gained from these experiments it was necessary for further sampling to be carried out. A third group of sampling was carried out running the same experiments in 'reverse order', sampling during the highest disturbance activity first and in an undisturbed environment last. As in the second sample group, two repeat samples for each of the three activities

detailed in Table 3.2 were taken but on this occasion sampling was carried out in parallel with a LN5 laser sampler which ran throughout the sampling period. The bioaerosol concentrations measured over time are shown in Figure 4.39.

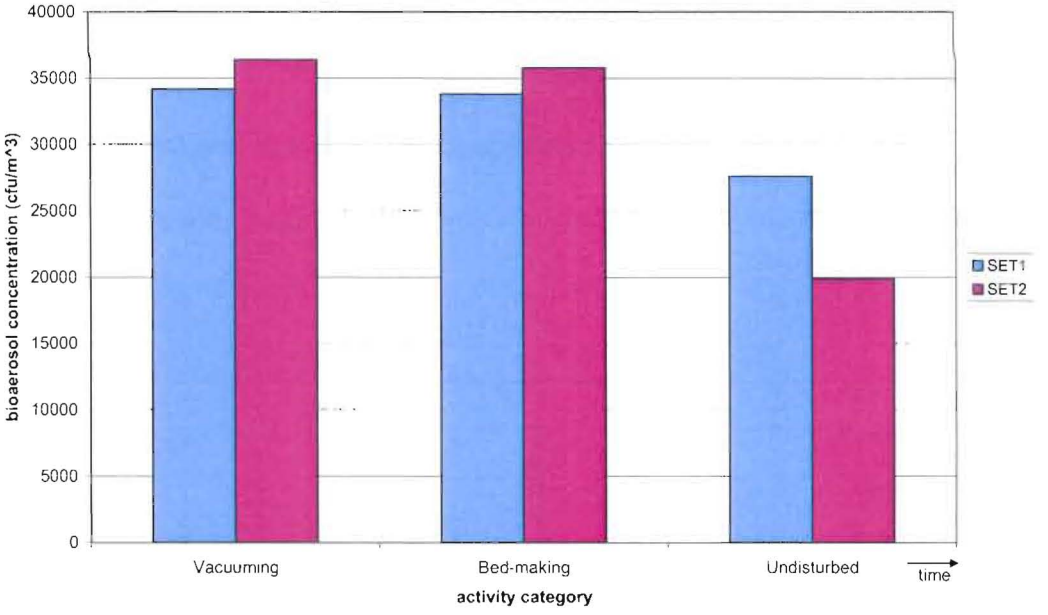


Figure 4.39: Bioaerosol concentrations from Andersen sampling (group 3) in a damp bedroom during different disturbance activities – set 1 and set 2 are repeats of the same activity category (n=1).

These results agree with the hypothesis that measured bioaerosol concentrations are related to the level of human activity in the environment at the time of sampling. Vacuuming, the most vigorous activity, resulted in the highest bioaerosol concentrations with undisturbed conditions again giving the lowest. Overall, mean bioaerosol concentrations during bed-making and non-disturbance were much higher from Group 3 (34795 and 23744 cfu/m^3, respectively) than in Group 1 (3721 and 438 cfu/m^3, respectively). This indicates that there remains an observed effect of time on the sampling results, with continuing disturbance

activity causing increased total airborne particulate concentrations even during periods of calm.

The effect of human activity on the bioaerosol concentrations measured by the Andersen sampler can be more clearly seen in Figure 4.40. The bioaerosol data from the third group of samples (shown previously in Figure 4.39) has been plotted against total airborne particulate data that was measured simultaneously using the LN5 laser sampler.

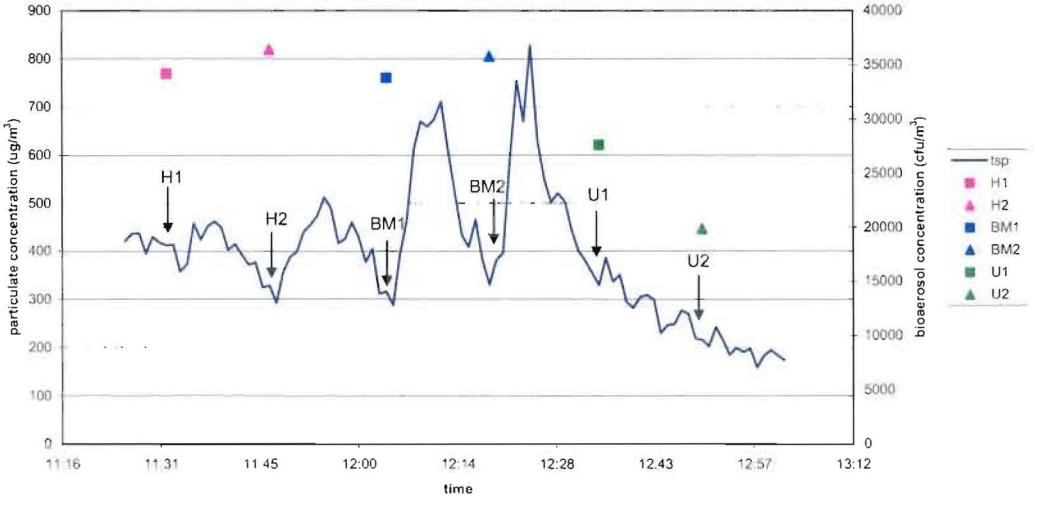


Figure 4.40: comparison of the effect of different disturbance activities on Andersen and LN5 sampling results in a damp bedroom with visible mould growth [H – hoovering, BM – bed-making, U – undisturbed].

The concentration of total suspended particulates (TSP) shows distinct fluctuations during each of the periods of disturbance activity. These variations are reflected by corresponding variations in measured bioaerosol concentrations. The results shown in Figure 4.40 show vacuuming to have resulted in the highest

bioaerosol concentrations, despite the fact that bed-making caused the highest increases in total airborne particulate concentrations. This agrees with the bioaerosol data shown in Figures 4.37, 4.38 and 4.39 which show measured bioaerosol concentrations to be highest during vacuuming.

In addition to merely measuring numbers of microorganisms, the main fungal species cultured were also identified and compared to those species that were present as visible growth on the damp walls. It is important to note that equivalent species were identified on the walls and the culture plates. Only a small proportion of the total number of viable microorganisms isolated in each sample were bacterial. The majority of isolates were fungal and the main species isolated was *Penicillium*, in addition to a smaller number of sterile mycelia. This shows a clear relationship between the presence of spore-producing species in a domestic environment and the detection of these spores in air samples taken from that environment.

4.13 The effect of sampling height on the total efficiency of the Andersen microbial sampler

To examine the effect of sampling height on Andersen sampler collection efficiency, the total numbers of microorganisms collected on all six plates as well as the individual numbers on each stage of the Andersen sampler for each house, were examined for both NA and MEA (Figure 4.41 and 4.42). Statistical analyses of the data were then performed using the Mann-Whitney test (Appendix 10).

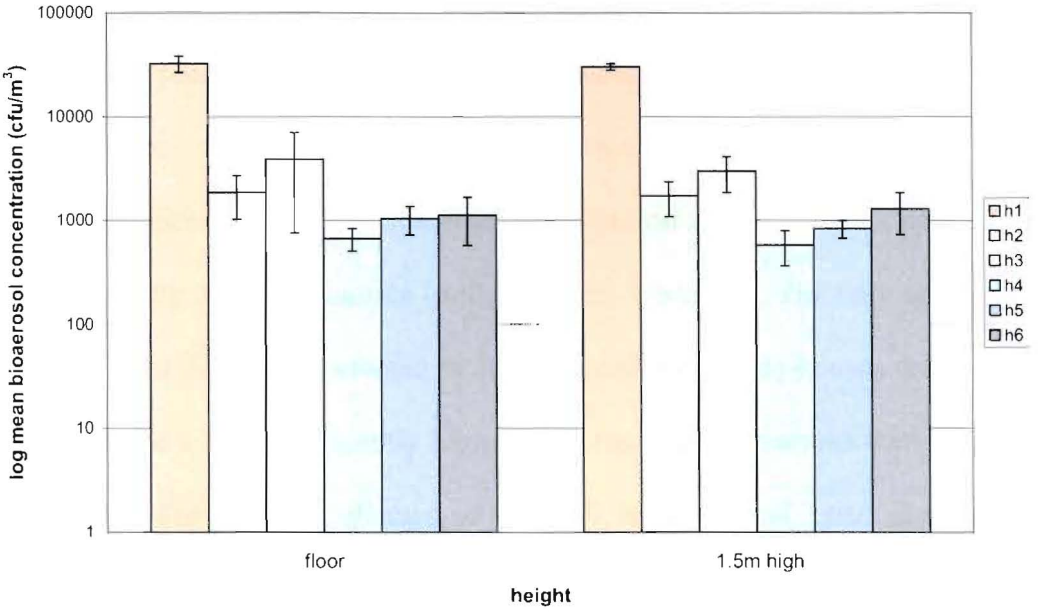


Figure 4.41: Log mean bioaerosol concentrations measured by the Andersen sampler on NA at 37°C at two sampling heights (error bars show standard deviation, n=9 for all houses except h2 where n=3)

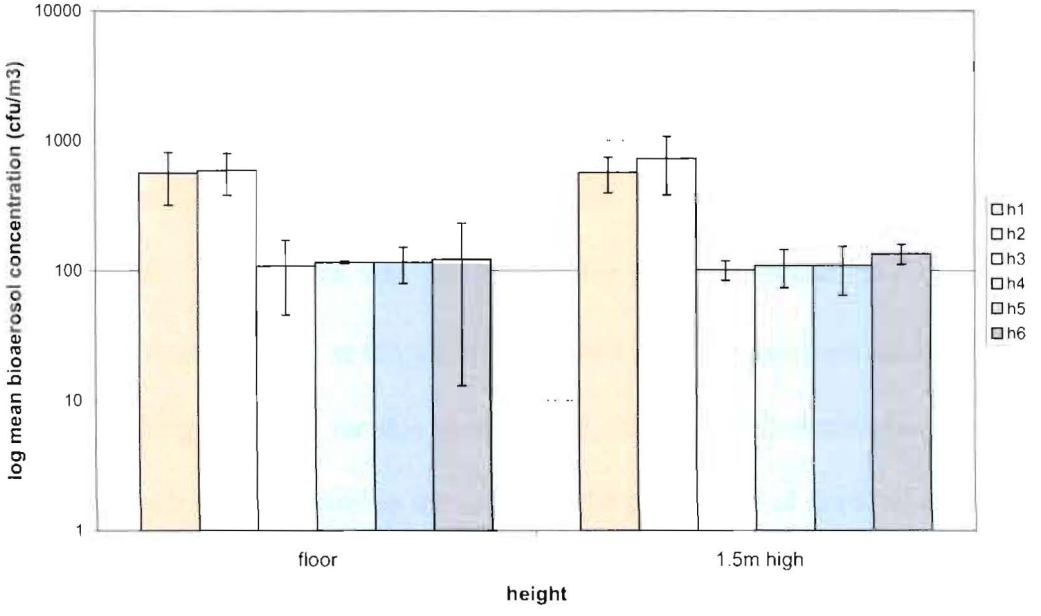


Figure 4.42: Log mean bioaerosol concentrations measured by the Andersen sampler on MEA at 37°C at two sampling heights (error bars show standard deviation, n=9 for all houses except h2 where n=3)

There were no significant differences ($p>0.05$, Mann-Whitney) between the total viable counts taken on NA or MEA from floor and 1.5 m high samples for any of the six houses. Additionally, there were no significant differences in the mean bioaerosol concentrations detected at either height on any of the six stages of the sampler for any of the six houses ($p>0.05$, Mann-Whitney). The only area where any significant difference between samples is seen is between houses themselves. On NA, house 1 had significantly higher bioaerosol concentrations than the other five houses. For samples collected on to MEA, houses 1 and 2 had significantly higher total bioaerosol concentrations than the remaining four houses, but they are not statistically different from each other.

The effect of sampling height on the proportion of each species type collected on each stage of the Andersen sampler was then examined. Species were categorised based on their morphological characteristics (Appendix 16) for analysis in SPSS and subsequently identified using the methods previously described. The data were selected for analysis according to medium, temperature, house number and plate number. No analysis was carried out for plates incubated at 25°C as all 25°C plates were sampled at the same height of 1.5 m, allowing no assessment to be made of height effects for this temperature. Rather than examining the total number of each species found in the samples, the proportion of the total count that each species comprised was examined, to allow for differences in total viable counts between different samples. Analyses were carried out to examine the proportion of each species found on each of the six stages of the Andersen

sampler at both sampling heights. Each house was analysed individually as well as in a combined data set comprising all six houses.

In the combined data set, using species data from all six houses, there was no relationship between the height the sample was taken at and the types and numbers of species collected on each stage, for either nutrient agar or malt extract agar.

On NA at 37°C, the Mann-Whitney test showed there to be no significant differences ($p>0.05$, two-tailed) in the proportions of any species between samples taken at floor height and those taken at 1.5 m high on any of stages 1-6.

On MEA at 37°C when species data for all 6 houses was combined, there were again no significant differences in the proportions of any species between the samples taken at the two sampling heights on any of stages 1-6 ($p>0.05$, Mann-Whitney, two-tailed).

The Mann-Whitney test was then performed for each stage number for individual houses where samples had been taken onto NA at 37°C. No significant differences between heights were observed except for those detailed in Table 4.4.

When the analysis was carried out on individual houses for samples on MEA at 37°C for each stage number, no differences between heights were observed except for those listed in Table 4.5.

Table 4.4: Species for which there was significant difference (Mann-Whitney, $p<0.05$) in proportion between samples taken at floor height and at 1.5 m high on NA.

House	Stage	Result	Likely explanation
2	2	▪ The proportion of round cream shiny colonies (*Staphylococcus epidermidis* – isolated from human skin) was significantly higher in samples taken at floor height.	▪ During sampling, small child had placed hands over the inlet of the Andersen sampler, whilst it was placed on the floor increasing the proportion in floor samples.
2	3	▪ The proportion of flat white dry colonies (*Bacillus licheniformis* – common in soil/water) was significantly higher in samples taken at floor height.	▪ Trampled into carpet from outdoors. Localised disturbance caused by setting up sampling equipment is likely to have been sufficient to re-suspend the cells in the vicinity of the sampler, but not enough to distribute them throughout the room.
6	2	▪ The proportion of orange lumpy dry colonies (*Actinomadura* – isolated from soil) was significantly higher in samples taken at 1.5 m high.	▪ Trampled into the house on occupant's shoes and deposited onto the carpet. Recent vacuuming of the carpet may have resulted in them becoming airborne.
6	6	▪ The proportion of smooth orange shiny colonies (*Bacillus* spp – common soil organism) was significantly higher in samples taken at 1.5 m high.	▪ Recent vacuuming of the carpet is likely to have resulted in cells becoming airborne.

Table 4.5: Species for which there was significant difference (Mann-Whitney, $p<0.05$) in proportion between samples taken at floor height and at 1.5 m high on MEA.

House	Stage	Result	Explanation
5	2	▪ The proportion of beige smooth shiny colonies (*Stenotrophomonas Maltophilia* - isolated from soil, water, vegetation) was significantly higher in samples taken at floor height.	▪ Trampled into carpet from outdoors. Localised disturbance caused by setting up sampling equipment could have been sufficient to re-suspend cells in the vicinity of the sampler, but not enough to distribute them throughout the room.
5	6	▪ The proportion of beige smooth shiny colonies (*Stenotrophomonas maltophilia*) was significantly higher in samples taken at floor height.	▪ Trampled into carpet from outdoors. Localised disturbance caused by setting up sampling equipment could have been sufficient to re-suspend cells in the vicinity of the sampler, but not enough to distribute them throughout the room.
6	3	▪ The proportion of beige smooth shiny colonies (*Stenotrophomonas maltophilia*) was significantly higher in samples taken at floor height.	▪ Trampled into carpet from outdoors. Localised disturbance caused by setting up sampling equipment could have been sufficient to re-suspend cells in the vicinity of the sampler, but not enough to distribute them throughout the room.
6	4	▪ The proportion of fine white fluffy colonies (sterile mycelia) was significantly higher in samples taken at 1.5 m high.	▪ Recent vacuuming of the carpet is likely to have resulted in spores from carpet becoming airborne.

Although there were several cases where significant differences in the proportion of species found at different heights were observed, these can be explained. In addition to the reasons stated in Tables 4.4 and 4.5, these differences may also have been related to the ability of particular species to remain airborne. The likelihood of individual species becoming airborne will vary according to the specific shape and size of each cell type.

In general, however, it can be concluded that sampling height had no effect on the type and number of microbial species collected by the Andersen sampler, either as a whole or on its individual stages.

4.14 The effect of incubation temperature on the numbers and proportions of species collected by the Andersen microbial sampler

The effect of incubation temperature on the total number of species and the proportion of each species type collected on the six stages of the Andersen sampler was examined. The data were selected for analysis according to medium, house number and plate number. All data was from samples collected at 1.5 m high as these were the only samples that were incubated at two different temperatures. These temperatures were 25°C and 37°C with all incubations carried out for 72 h. Species were categorised based on their morphological characteristics for analysis in SPSS and subsequently identified. Not all of these microorganisms were successfully identified and therefore only known organisms have been reported in the final results tables (Appendix 16). Where an unidentified species was present in significant abundance, however, this has been noted accordingly. In addition to examining the total number of each species found, the proportion of the total count that each species comprised was examined, to allow for differences in total viable counts between samples. The proportions of each species as well as the total count found, combined for all six stages of the Andersen sampler, were compared for the two temperatures, for both NA and MEA.

Analyses were carried out to examine the total number of species found at both incubation temperatures on each medium type. Each house was analysed individually as well as in a combined data set including all six houses.

On NA, for five of the six houses, there was no significant difference ($p > 0.05$, Mann-Whitney, Appendix 11) between 25°C and 37°C in the total numbers of microorganisms found on the six Andersen stages (Figure 4.43). For house 5, however, significantly more organisms were cultured at 25°C than at 37°C ($p < 0.05$, Mann-Whitney).

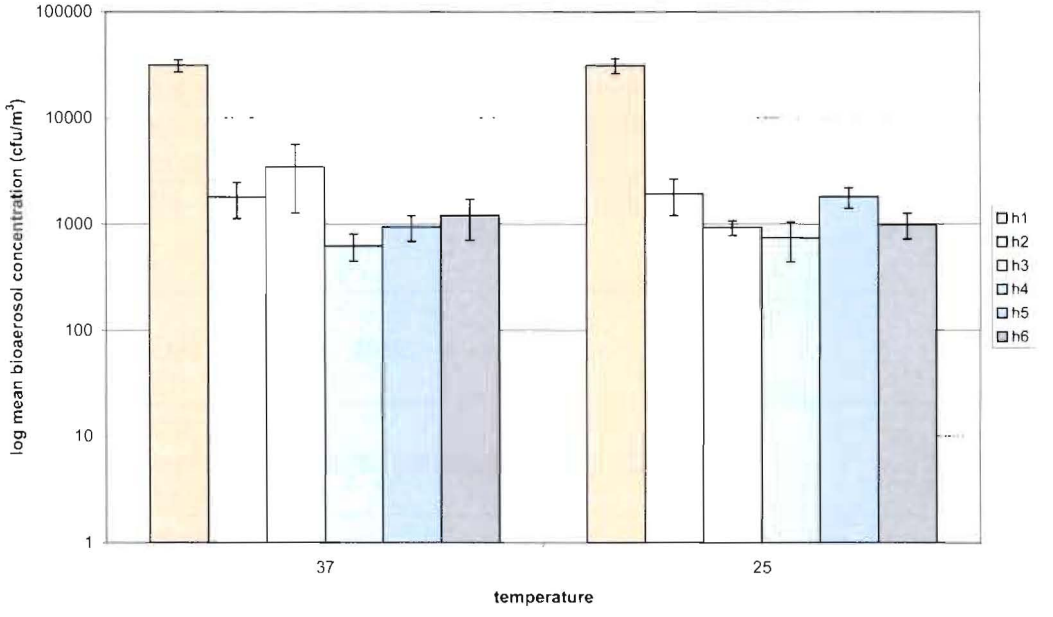


Figure 4.43: Log mean bioaerosol concentrations measured by the Andersen sampler on NA at 1.5 m high at 37°C and 25°C (error bars show standard deviation, n=21 for 37°C and n=9 for 25°C for all houses except house 2 where n=7 for 37°C and n=3 for 25°C)

When the same analyses were carried out on the combined data from all six houses (Appendix 12), there was no significant difference between the total numbers of microorganisms that grew on NA plates incubated at 25°C and those at 37°C ($p > 0.05$, Mann-Whitney). There were however, significant differences in the proportions of several individual species at the two growth temperatures and these are detailed in Table 4.6.

Table 4.6: Species for which there was a significant difference (Mann-Whitney, $p<0.05$) in proportion between samples incubated at 25°C and at 37°C on NA.

Significantly higher proportion at 25°C	**Significantly higher proportion at 37°C**
Arthrobacter spp (soil bacteria, optimum growth temperature 25°C)	*Staphylococcus* spp (bacteria commonly isolated from humans)
Cryptococcus spp (yeast commonly isolated from environmental samples)	*Micrococcus luteus* (bacteria commonly found on human skin)
Sterile mycelia (commonly isolated from the environment)	
Acremonium (fungal species isolated from plant debris and soil)	
Penicillium spp (fungus widespread in nature, common in environmental samples)	
Cladosporium spp (fungus widespread in nature, common in environmental samples)	
Rhizopus spp (fungus common in environmental samples)	
Streptomyces spp (commonly isolated from soil)	

On MEA, five of the six houses showed a significant difference ($p < 0.05$, Mann-Whitney) in the total numbers of microorganisms found on the six Andersen stages between 25°C and 37°C (Figure 4.43). There were significantly more microorganisms isolated at 25°C than at 37°C for each of house 1, 3, 4, 5, and 6 (Appendix 11). For house 2, however, there was no difference between the two growth temperatures ($p > 0.05$, Mann-Whitney).

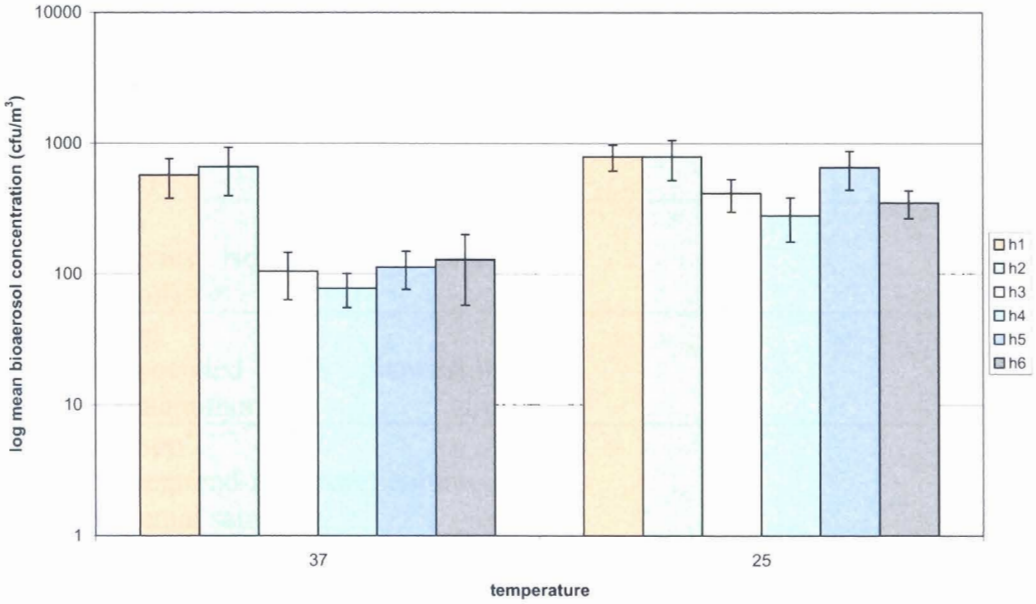


Figure 4.44: Log mean bioaerosol concentrations measured by the Andersen sampler on MEA at 1.5 m high at 37°C and 25°C (error bars show standard deviation, n=21 for 37°C and n=9 for 25°C for all houses except house 2 where n=7 for 37°C and n=3 for 25°C)

When the same analyses were carried out on the combined data from all six houses, there was a significantly higher number of total microorganisms isolated on MEA at 25°C than at 37°C ($p < 0.05$, Mann-Whitney). Furthermore, there

were significant differences in the proportions of individual species at the two growth temperatures (Appendix 12) and these are detailed in Table 4.7.

Table 4.7: Species for which there was a significant difference (Mann-Whitney, $p<0.05$) in proportion between samples incubated at 25°C and at 37°C on MEA.

Significantly higher proportion at 25°C	**Significantly higher proportion at 37°C**
Arthrobacter spp (soil bacteria, optimum growth temperature 25°C)	*Staphylococcus epidermdis* (bacteria commonly isolated from human skin)
Actinomadura spp (commonly isolated from soil)	*Pseudomonas oryzihabitans* (bacteria commonly found on human skin)
Sterile mycelia (commonly isolated from the environment)	
Acremonium (fungal species isolated from plant debris and soil)	
Fusarium spp (fungus associated with plants/soil, common contaminant).	
Penicillium spp (fungus widespread in nature, common in environmental samples)	
Cladosporium spp (fungus widespread in nature, common in environmental samples)	
Rhizopus spp (fungus common in environmental samples)	

4.15 The relationship between domestic bioaerosol concentrations measured using different sampling methods and the reported health effects experienced by house occupants

For each of the seven houses selected from the Damp Homes study mean bioaerosol concentrations collected by both the Andersen and Omega viable samples were plotted against mean airborne particulate concentrations measured by the LN5 laser sampler. In each case the bioaerosol concentrations for both samplers accurately reflected the particulate concentrations in the air at that time. An example of the pattern of data is shown for house 4 in Figure 4.45. It was also confirmed that the Omega and Andersen samplers achieve comparable results when sampling on to NA, with no significant difference observed between results ($p > 0.05$, Mann-Whitney, Appendix 13).

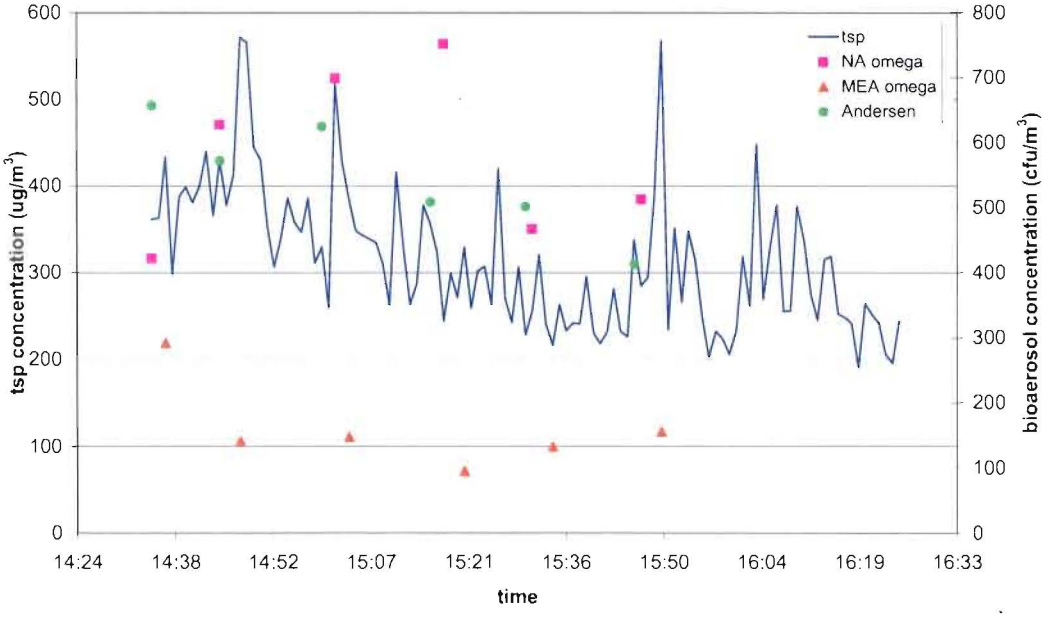


Figure 4.45: Simultaneous measurement in House 4 of bioaerosol concentrations using the Andersen and Omega samplers and particulate concentrations using the LN5 laser monitor.

In addition, for both the Andersen and Omega samplers collecting on to NA there was a highly significant correlation ($p < 0.01$, Spearman's rho) between mean bioaerosol and mean PM 1 concentrations (Appendix 13). This was also seen on MEA for the Omega sampler. No Andersen samples were taken using MEA so no analysis can be performed for this. Additionally, for Omega samples on MEA there was a highly significant association ($p < 0.01$, Spearman's rho) between mean bioaerosol concentrations and PM 2.5 levels. This association was also found on NA for both the Andersen and Omega samplers but was less strong ($p < 0.05$, Spearman's rho). A further correlation was found for the Andersen sampler between TSP concentrations and bioaerosol levels ($p < 0.05$, Spearman's rho).

It was found that there was no statistically significant difference between the bioaerosol concentrations measured for samples that had been disturbed using the fan agitator and those that had not ($p > 0.05$, Mann-Whitney), either for samples collected using the Andersen sampler or using the Omega AIRTEST (Appendix 14). Data from these two sets of disturbed and undisturbed samples were subsequently combined for further analyses.

Mean bioaerosol concentrations as measured by the Andersen sampler were examined for each of the seven houses (Figure 4.46). It can be seen that the bioaerosol concentrations measured in house 6 were significantly higher than for any of the other six houses.

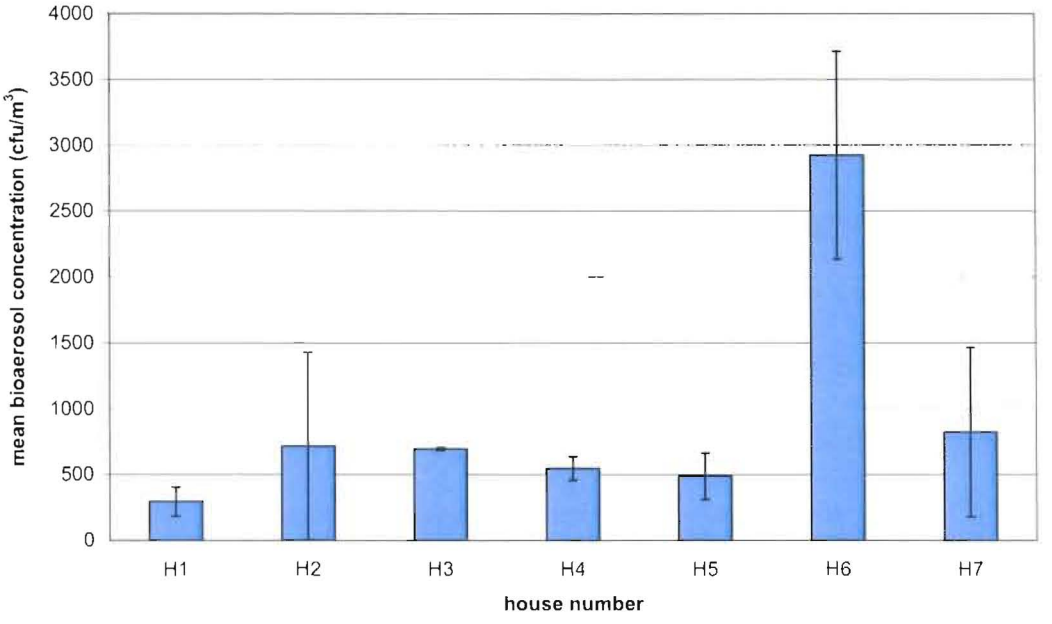


Figure 4.46: Mean bioaerosol concentrations in seven houses as measured by the Andersen sampler (error bars show standard deviations, n=6)

It was concluded that both the Andersen and Omega samplers provide an accurate (and directly comparable) picture of the fluctuating bioaerosol concentrations likely to be encountered in a domestic environment.

The results of the questionnaire administered in the DoH study were analysed and compared against the bioaerosol data collected for each house in this work. Initial analyses of the data to examine the number of reported health symptoms with respect to actual measured bioaerosol concentrations showed no relationship between the levels of bioaerosols and the number of health effects experienced. The number of individual measures of bioaerosol concentration was too great for any meaningful pattern to be identified between a particular concentration and the number of symptoms so the data had to be analysed in a different way. The bioaerosol concentrations measured by the Andersen and Omega samplers were

subsequently categorised as high, intermediate and low, representing bioaerosol concentrations of < 10000 cfu.m^{-3}, < 1000 cfu.m^{-3} and < 200 cfu.m^{-3}, respectively. Plotting of the data showed evidence of an association between the level of measured bioaerosol concentrations and the number of total symptoms reported. The data showed that homes which had a higher measured concentration of bioaerosols had a higher number of total symptoms reported by house occupants. For example, the occupant of house 6, which showed the highest bioaerosol concentrations, reported the highest number of total symptoms (21) of all the study participants (Figure 4.47).

No statistically significant relationships could be found to support this observation, however, as n numbers for the health data of house occupants were too low to allow valid tests to be performed. Visually, a better relationship was found between high bioaerosol concentrations and a higher number of reported symptoms when the bioaerosol concentrations were categorised only as low (0 – 500 cfu.m^{-3}) or high (< 500 - < 10000 cfu.m^{-3}). However, statistical analyses comparing the two sets of data found these differences not to be significant ($p > 0.05$, Mann-Whitney).

The responses to the health questionnaire were also sorted into types of symptoms rather than the total number of symptoms. These categories were

1. Respiratory symptoms including wheeze, asthma, presence of a cough and breathlessness.
2. Skin symptoms such as flushed or problem skin.

3. Allergic symptoms including throat, eye and nose irritation and stuffy nose.
4. Rheumatic symptoms covering joint and muscle pain.
5. Head symptoms including headache, being unusually tired and difficulty in concentrating.
6. Other symptoms that do not clearly belong to any other group such as nausea, dizziness and palpitations.

These data were examined to compare the numbers and types of symptoms reported by the occupant of each of the seven study houses (Figure 4.47).

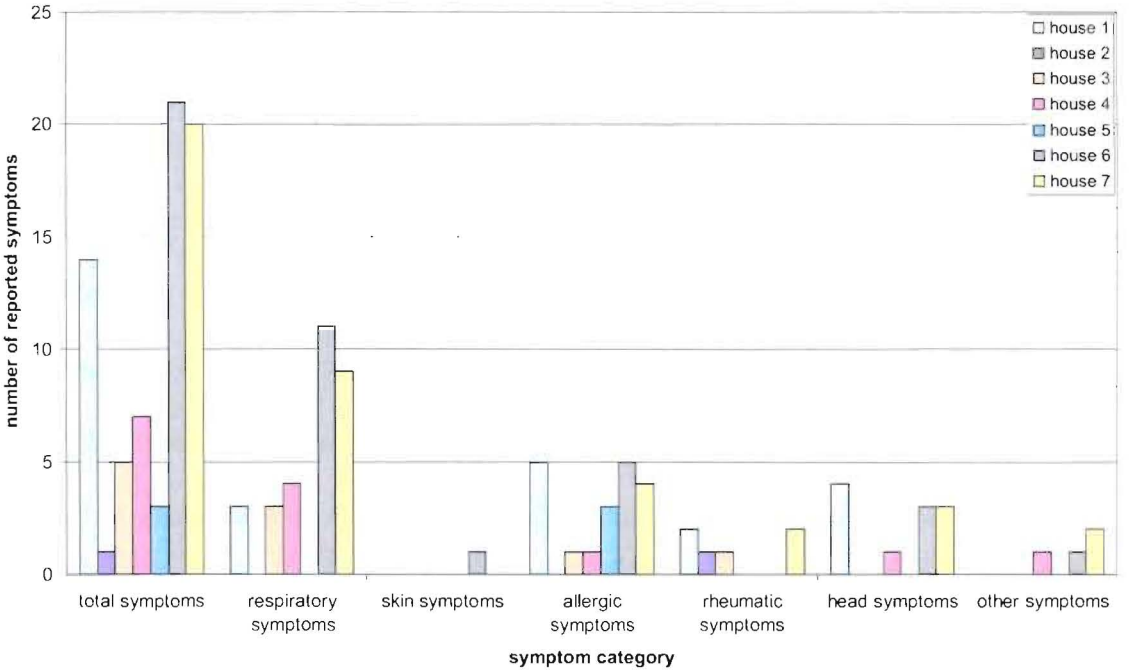


Figure 4.47: Comparison of numbers and types of symptoms reported by occupant of each study house.

The number of total symptoms reported ranged from just one (in house 2) to twenty-one (in house 6). Respiratory and allergic symptoms were generally reported in the highest numbers and with greatest frequency across the range of study houses, contrasting with only one report of problem skin (in house 6) recorded throughout the survey.

Using the data from Figures 4.46 and 4.47 it can be seen that although house 6 (with the highest bioaerosol concentrations) had the highest number of total reported symptoms, this was not always true for specific groups of symptoms e.g. head symptoms. Also, house 1, which had the lowest measured bioaerosol concentrations, did not correspondingly have the lowest number of symptoms.

Analyses of the data when bioaerosol concentrations for each house were classified as either low or high were carried out. Graphs (not shown) showed that there was a greater number of each group of symptoms reported at high bioaerosol concentrations than at low concentrations. Statistical analyses, however, found that none of these relationships were significant ($p > 0.05$, Mann-Whitney). It should be noted here that the numbers of cases being analysed are very low, sometimes using only a single value compared against another. Under these conditions it is unlikely that significant associations can be found in the data, even if they are present.

Ideally, these results would have been used to determine which parameter provides the best indication of the actual and perceived health effects collected

during the DoH study. It was hoped to identify the best way to monitor bioaerosols in the indoor environment with regard to the health effects predicted. However, beyond the initial analyses it was not possible to perform meaningful statistical tests as, having n numbers of only 3 – 5, the Mann-Whitney was often not a powerful enough test to find associations in such small data sets. Additionally, when attempting to perform Crosstabs analyses to look for an association between groups of symptoms and levels of bioaerosol concentration, outcomes were reported by SPSS as invalid.

4.16 Comparison of reproducibility of results between different sampling methods

Sampling was carried out in six houses, measuring bioaerosol concentrations using a range of samplers and techniques. The results obtained using each method and their comparability with each other are discussed in sections 4.16.1 to 14.16.3.

4.16.1 AGI-30 impinger measured bioaerosol concentrations

The viable results obtained from plating out the collection fluid from the AGI-30 impinger samples taken in each of the six houses were analysed. For each house it was possible to combine the two sets of data obtained from the two separate impingers, as it had been shown that the results obtained from each were not statistically significant from the other ($p > 0.05$, Mann-Whitney). Taking each house separately, the data was examined for total viable concentrations on NA and

MEA at 25 °C and on NA and MEA at 37°C. As seen with so much of the bioaerosol data collected in this study there was huge variability in the results which may have disguised any significant differences between samples. As it was, there were no significant differences ($p > 0.05$, Mann-Whitney) between the mean bioaerosol concentrations on NA at 25°C and 37°C (Figure 4.48) or on MEA at 25°C and 37°C (Figure 4.49).

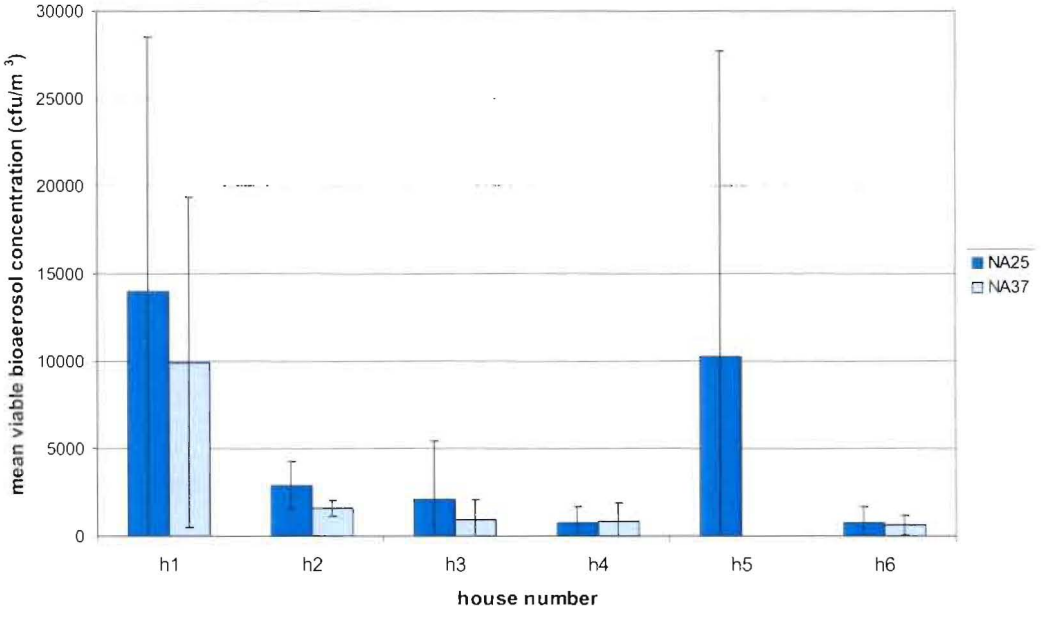


Figure 4.48: Mean bioaerosol concentrations measured by the AGI-30 liquid impinger when plated on to NA and incubated at 25°C and 37°C (error bars show standard deviations, house1 n=6, house 2 n=2, house 3 n=4, house 4 n=6, house 5 n=4, house 6 n=6).

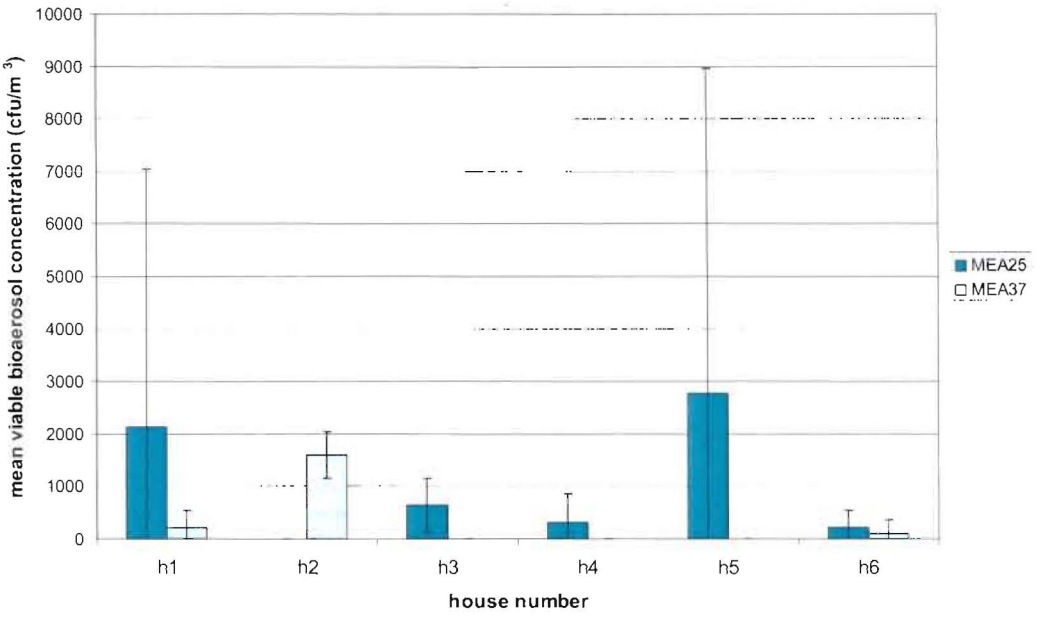


Figure 4.49: Mean bioaerosol concentrations measured by the AGI-30 liquid impinger when plated on to MEA and incubated at 25°C and 37°C (error bars show standard deviations, house1 n=6, house 2 n=2, house 3 n=4, house 4 n=6, house 5 n=6, house 6 n=6).

Unusually, there were no significant differences in mean concentrations between the two growth media.

Very few fungal colonies were found in the impinger samples from any of the six houses – even those with reported mould problems. No fungi were isolated from any sample, on any medium, at either temperature in houses 1 and 2. The few fungal results that were recorded are detailed in Table 4.8.

Table 4.8: Impinger samples that contained viable fungal colonies following air sampling in six houses.

House number	Sample number & conditions	Number & type of fungal colonies
3 (Set 3)	- MEA, 25°C - MEA, 37°C	- 2 unknown with sterile mycelia - 1 *Penicillium*
4 (Set 3)	- NA, 37°C - MEA, 25°C	- 1 unknown with sterile mycelia - 1 *Cladosporium*, 1 unknown with sterile mycelia
5 (Set 3)	- NA, 25°C	- 2 *Cladosporium*
6 (Set 1)	- NA, 25°C	- 1 unknown (grey-brown)
6 (Set 3)	- NA, 25°C - MEA, 25 °C	- 3 *Cladosporium* - 1 *Cladosporium*, 1 unknown with sterile mycelia

These results indicate that, although measured fungal concentrations were low, in order to increase the likelihood of isolating fungal species, impinger samples should be incubated at 25°C.

When compared directly with concurrent Andersen sampling results it was seen that there was no significant difference ($p > 0.05$, Mann-Whitney) in the mean bioaerosol concentrations measured by the two methods for either NA or MEA at either 25°C or 37°C (Figure 4.50)

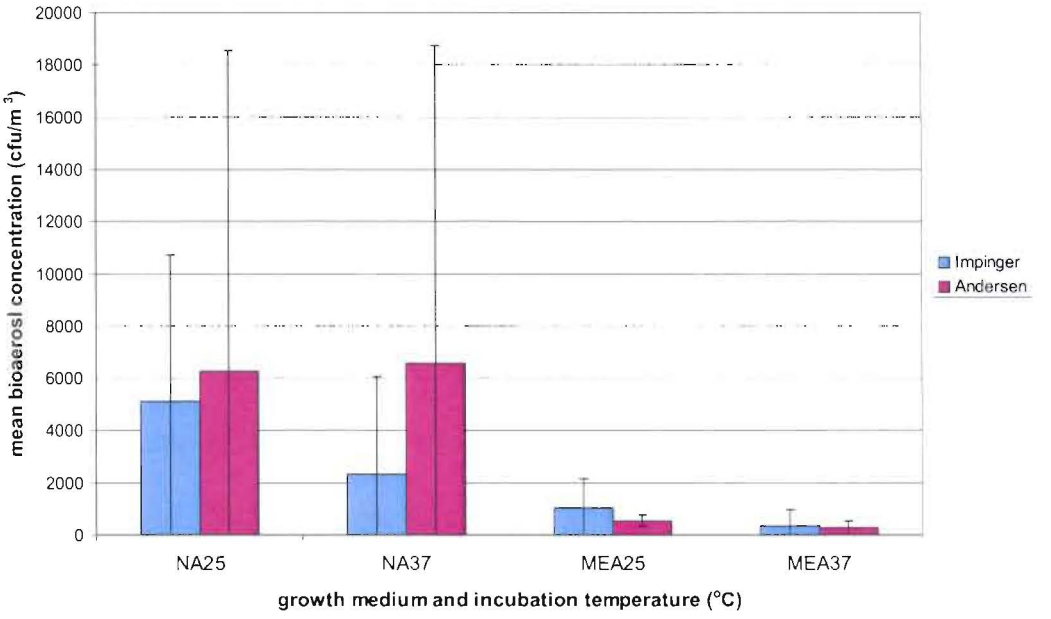


Figure 4.50: Compared sampling efficiencies of the Andersen sampler and the AGI-30 impinger on NA and MEA at 25°C and 37°C (error bars show standard deviations, n=28).

It was concluded that the AGI-30 all-glass impinger collects bioaerosol concentrations that are directly comparable with those collected by the Andersen viable sampler. However, the low numbers of fungal cells seen in the impinger results compared to those from the Andersen sampler were a cause for concern.

4.16.2 Measured bioaerosol concentrations from filter samples

Filter samples were collected in each house and the filter wash fluid from each sample was stained with acridine orange for microscopic examination under UV. It was hypothesised that it would be possible to calculate/predict the numbers of viable cells that could be expected to be found on each filter, based upon the numbers of viable colonies that were measured by the Andersen sampler, during

simultaneous sampling. This would, in effect, allow the sampling efficiencies of the two sampling methods to be directly compared. The number of viable colonies per litre of air sampled by the Andersen sampler was calculated and then used to predict the number of viable cells that could be expected to be found in filter samples where a volume of 360 l of air was sampled. Colonies counted on NA allowed the calculation of the expected number of viable bacterial cells in each filter sample. Expected numbers of viable fungi were calculated based on the number of fungal colonies counted on MEA for the relevant Andersen samples. Results showed that actual counts of viable bacteria, identified from the stained filter samples, were significantly higher than the expected counts that were calculated based on the Andersen sampling results (Figure 4.51). For House 1, actual viable bacterial counts (from the stained filter samples) were 26 times higher than the expected counts calculated from the Andersen samples. This contrasts with the results obtained for houses 2-6, where measured numbers of bacteria isolated from the filter samples were 150 – 300 times higher than the number predicted from the Andersen sampling results. House 1 had significantly higher concentrations of bacteria in the Andersen samples than the other five houses, which may account for the relatively small difference observed between actual and expected counts compared to the other samples.

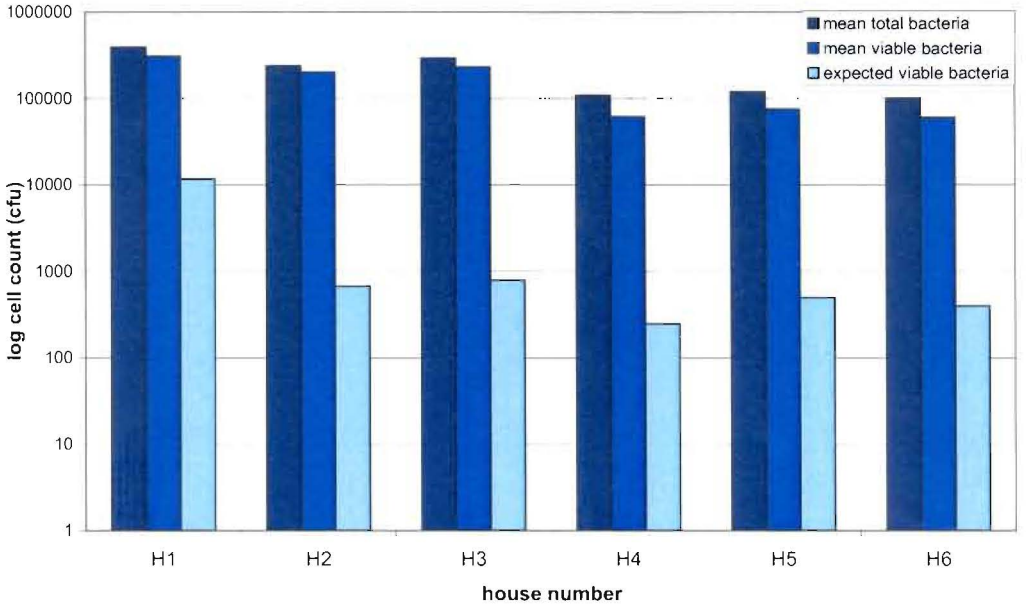


Figure 4.51: Log cell counts of actual versus expected viable bacterial cell counts for filter samples (n=2). Results compare the number of viable bacterial cells counted in stained filter samples with the predicted number of viable bacteria that would be in those samples, based on Andersen sampling results.

Actual counts of viable fungi in identified in stained filter samples were also found to be significantly higher than those expected, as calculated from the numbers of fungal colonies detected on MEA Andersen sampling plates (Figure 4.52).

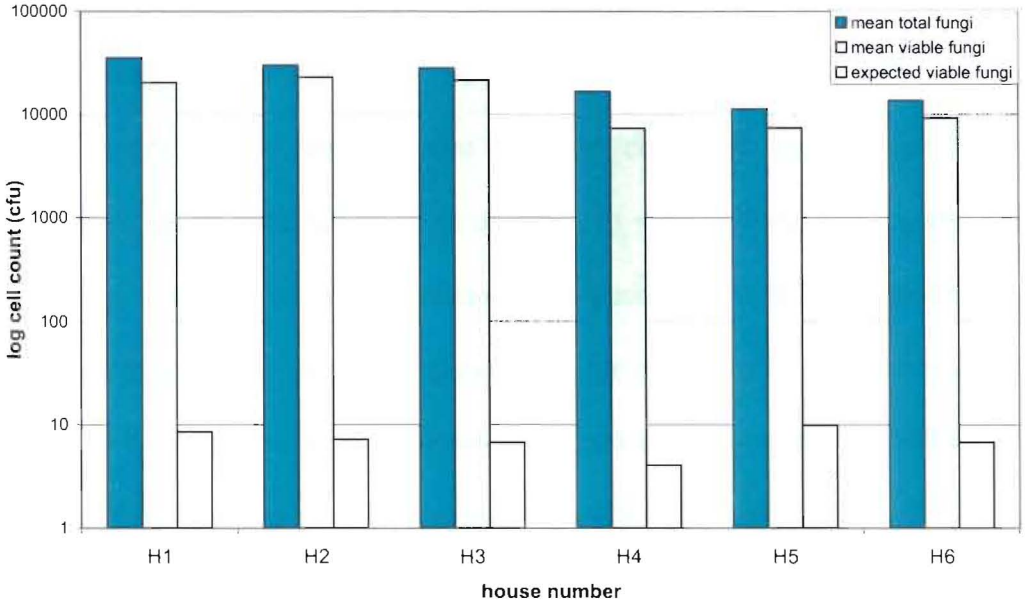


Figure 4.52: Log cell counts of actual versus expected viable fungal cell counts for filter samples (n=2). Results compare the number of viable fungal cells counted in stained filter samples with the predicted number of viable fungi that would be in those samples, based on Andersen sampling results.

Measured counts of viable fungi in the stained filter samples were 750 – 3190 times higher than the expected levels, calculated based on the Andersen sampling results.

Data from acridine orange staining of filter samples that were run alongside Andersen samples were analysed to examine the comparability of sampling results achieved using the two methods (all cases n=16). See Appendix 15. Results were investigated to determine whether the results from one method would allow the accurate prediction of the results that would be obtained using the other. The total bacterial and viable bacterial numbers from acridine orange staining of wash fluid from the filter samples had a highly significant correlation with the concentration

of bacteria in Andersen samples on NA following incubation at 37°C for 72 h ($p < 0.01$, Spearman's correlation). The number of total bacterial cells in acridine orange stained filter samples also strongly correlated with viable bacteria concentrations in Andersen samples on MEA ($p < 0.01$, Spearman's correlation). There was a weaker, but still significant, relationship between the concentration of viable bacteria in the acridine orange samples and bacterial concentrations on MEA. No other meaningful relationships between the results from the stained filter samples and the Andersen sampling results were seen. A correlation was found between viable fungi concentrations in the acridine orange samples and bacterial concentrations on NA and MEA Andersen samples ($p < 0.05$, Spearman's correlation) but this was not thought to be a true relationship. It was therefore concluded that it is not possible to use the results from either method to directly predict or to generate a correction factor allowing the prediction of, the results that would be generated by the other method.

4.16.3 Measured viable microbial concentrations in surface samples of floor dust

The results from plating out serial dilutions of filter wash fluid from floor dust samples were not usable. Problems were encountered in the enumeration of the viable cell concentrations in each sample. The experimental method involved the plating of a serial dilution of each filter wash fluid on to agar plates. When examining the results and converting each plate count to a concentration of cells, each dilution resulted in the calculation of a different concentration of microorganisms. It was not possible even to compare dilutions of the same

sample as different viable cell concentrations were calculated depending on which dilution was used in the calculations. Therefore, no comparable results were achieved from these experiments to measure the viable microbial concentrations in surface dust.

Consequently, it was not possible to compare the viable bioaerosol concentrations measured in the surface samples with the results from the Andersen sampling and the acridine orange staining. It was found, however, that the species types isolated from floor dust samples correlated well with those isolated from the air.

5 DISCUSSION

5.1 Optimisation of Andersen sampler method

5.1.1 The effect on efficiency of using glass or plastic plates with the Andersen microbial sampler

When comparing the sampling efficiency of the Andersen sampler using different types of collection plates, the experimental results provided no evidence of a difference in the colony numbers resulting on glass plates to those on disposable plastic plates. The results from this study disagree with the findings of Andersen (1958) which recommend that plastic plates are unsuitable for use with the Andersen sampler. It was suggested that plastic plates yield colony counts approximately 20% lower than those achieved on the glass plates provided with the sampler. It was proposed that the reason for this was as a result of the electrostatic charge generated when using plastic plates, and the reported reduction that this has on the collection efficiency of the sampler.

On first inspection of the sample plates it appeared that there was a greater distribution of colonies impacted at the meniscus, where the agar met the plate wall, on plastic plates compared to the distribution on glass. An examination was made of the colony distribution in the outer 5mm of each plate compared to the distribution across the rest of the plate surface. As well as finding no evidence of

the retention of particles on the interior surfaces of plates, no evidence was found to support Andersen's finding (1958) that the electrostatic charges resulting from using plastic plates caused particle retention on the plate exteriors and the sampler walls. It was concluded that the use of plastic plates did not generate any effects significant enough to reduce the counts achieved on them, as compared to the custom glass plates designed for use with the Andersen sampler.

This is a particularly important result as it gives validity to the commonplace use of disposable plates throughout the area of Andersen sampling for bioaerosols. Reference to any investigation of the direct comparison of plastic and glass collection plates has not been found in the literature. These findings therefore justify the apparently contradictory use of disposable plastic plates throughout this study. The requirement for glass plates to be cleaned and re-sterilised between each use, and their numbers being in limited supply due to expense made them undesirable. Plastic plates were chosen to be used for all further experiments as the convenience afforded by having a large supply of disposable plates allowed multiple samples to be taken in short succession, with no adverse effect on the quality of samples recovered.

5.1.2 The effect of sampling time on the efficiency of the Andersen microbial sampler

Comparing sample run times of 2 minutes and 10 minutes on the collection efficiency of the Andersen sampler, no significant differences were found in the

concentration of microorganisms detected between the two sample times, across any of the four growth media tested (NA, TSA, PDA and MEA).

These results, however, disagree with data published by Folmsbee *et al.* (2000) where it is stated that "long run times may cause underestimation of bioaerosol content", due to the microbial sample being unable to survive the stress of long sampling times or the bioaerosol being lost due to re-entrainment and/or wall losses. The Folmsbee study examined the effect of sample run times that ranged from 1 minute to 6 minutes, in 1 minute increments, on the concentration of total culturable bacteria on TSA. It was concluded that a sample time of 2 minutes was the optimum run length for obtaining viable bacterial samples. Rather than the standard 6-stage Andersen sampler, however, the Folmsbee study used a modified 2-stage version, where the first and second stages had cut-off diameters of 7 and 0.65 μm, respectively. These are equivalent to the first and sixth stages of the 6-stage Andersen sampler. The manufacturers (Graseby-Andersen Ltd, Georgia) produce a 2-stage sampler, designed to separate particles into respirable and non-respirable fractions, using a cut-off diameter of 0.8 μm. It remains unclear whether the experimental method used by Folmsbee *et al.* (2000) was entirely valid as 45 ml of agar was used rather than the 27 ml recommended for the 6-stage sampler or the 20 ml recommended for the 2-stage version.

Another factor that may have affected the results reported by Folmsbee *et al.* (2000) is the air humidity at the time of sampling. If humidity was low then it is

likely that the sample would have become desiccated, and probably no longer viable, in a shorter time than would normally be expected at higher humidities.

Heinsohn (1999) compared the effect of sample times of 1 and 2 minutes, on the total viable counts achieved in residential environments. It was concluded that 1 minute samples generated less reliable data than samples run for 2 minutes. In another study, however, Gillespie *et al.* (1981) performed Andersen sampling at several wastewater treatment plants. In this study, sample times of 2 to 10 minutes were used and no effect of sample time on the sampled bioaerosol concentrations was noted.

Future sampling in this study was elected to be performed using a run time of 10 minutes. Other research has questioned the use of sampling times as long as this, with regard to concerns of sample desiccation and plate overloading. This was not a major concern here as the indoor environments from which the samples were to be taken had only mild to moderate levels of contamination, particularly when compared to the majority of heavily contaminated environments in which the other documented sampling has taken place e.g. agricultural settings, waste recycling facilities, composting plants.

5.1.3 The effect of growth medium on the efficiency of the Andersen microbial sampler

Of the four agar media selected for comparison in this study, two are designed for the selective growth of yeasts and moulds. Malt extract agar and potato dextrose

agar are for the isolation and enumeration of yeasts and moulds, while inhibiting many bacteria. Nutrient agar and tryptone soy agar are both general purpose media for the growth of a wide variety of nutritionally non-demanding organisms, commonly used for bacterial samples. Therefore, the significant differences observed between the counts obtained on NA and PDA, and those between NA and MEA were not unexpected, as NA is more suited to bacterial growth, while MEA and PDA are designed for fungal growth. This may also explain the fact that counts on NA (and to a certain extent, TSA) were highest, as there tends to be larger proportion of bacteria than fungi naturally present in the air. It is standard in most studies where the numbers of a particular species are of interest that the growth medium is selected to encourage the growth of that organism.

For future sampling, rather than using all four growth media, two were chosen to be used in the standard sampling protocol. Nutrient agar was selected to obtain viable bacterial counts and malt extract agar was selected for viable fungi. This is supported by the fact that MEA is the growth medium recommended by the American Conference of Governmental Industrial Hygienists (ACGIH) for aeromycological sampling (Burge *et al.*, 1987; Burge & Otten, 1999; Smid *et al*, 1989). NA is also regarded as an effective general purpose medium for the growth of bacteria from air samples (Burge & Otten, 1999; Jensen & Schafer, 1998).

The selection of a growth medium for the culture of airborne microorganisms is of major importance, determining the resultant concentrations and species that can

be isolated from a sample, and must be a key consideration in the development of any bioaerosol sampling regime.

5.1.4 The effect of incubation temperature on the efficiency of the Andersen microbial sampler

The preliminary experiments carried out to optimise the Andersen sampler sampling methodology showed that, for a 72 h incubation period, an incubation temperature of 25°C gave higher total viable counts than those achieved using an incubation temperature of either 30°C or 37°C. This is a significant result with respect to other studies employing the Andersen sampler where higher incubation temperatures of 35-37°C are commonly used, even for environmental samples. There appears to be no valid scientific reason for this other than the fact that the resulting counts will be obtained faster than at lower temperatures.

The fact that this study showed 25°C to obtain the highest counts when plates were incubated for 72 hours, suggests that studies where plates are incubated between 30°C and 37°C (e.g. Gillespie *et al.*, 1981, Jensen *et al.*, 1992, Morring *et al.*, 1983, Macher *et al.*, 1997) are likely to obtain misleadingly low measures of bioaerosol concentration.

For future sampling, however, 30°C was chosen as the optimum incubation temperature for bioaerosol samples taken during this study, compromising between the numbers of colonies grown and the speed of those colonies' growth in the 72 hour incubation time.

The effect of incubation temperature on the species composition of samples is discussed further in Section 5.15.

5.2 The Omega AirTEST air sampler

The Omega AIRTEST sampler is not a well characterised sampler and there is no reference to its previous use in the literature. The sampler was provided on loan for use in the first half of this study for evaluation purposes only, in an attempt to improve knowledge about its sampling performance. The results of this study have shown it to be a valuable piece of air sampling equipment for measuring bioaerosol concentrations in indoor air (Section 4.2). In terms of its collection efficiencies and measured bioaerosol concentrations, the Omega sampler compares favourably with the 6-stage Andersen viable sampler. It was also found to accurately reflect the airborne concentrations of particulate matter in indoor environments.

5.3 The effect of season and wind speed and direction on Andersen and Omega sampling results

Season has been well documented to influence the results of bioaerosol sampling, particularly in outdoor samples (Madelin, 1984; Verhoeff *et al.*, 1992; Shelton *et al*, 2002). It therefore seems logical to assume that weather factors such as wind speed and direction are likely to affect bioaerosol concentrations in an outside environment. It can be reasonably assumed that in an indoor environment where doors and windows to the external environment are effectively sealed, that wind

speed and direction are unlikely to have a significant effect on the measured bioaerosol concentrations indoors.

5.4 AGI-30 all-glass impinger

Despite the AGI-30 impinger being a standard reference sampler, the preliminary findings made in this study, taking impinger samples from the air in an indoor office, showed that it was difficult to detect bioaerosols in environments with low levels of contamination. Brachman *et al.* (1964) acknowledges that there may be situations where bioaerosol concentrations may be too low to be effectively sampled by an impinger. Cage *et al.* (1996) cited low collection efficiencies as a disadvantage of the AGI-30 over other sampling methods

The successful sampling of *Aspergillus* and *Penicillium* spores from above the surface of a heavily contaminated agar plate in the laboratory provided evidence that the sampling method was effective. Cage *et al.* (1996) refers to the point that using an impinger to collect particles into liquid may not be successful for many fungal spores, which tend to be hydrophobic in nature. This was not an apparent problem in this study as fungal colonies were grown from these impinger samples, as well as those taken in domestic environments. The effectiveness of this collection method, however, cannot be determined from these results. In order to examine the impinger's collection efficiency for fungal particles effectively, samples would need to be taken in an experimental environment where known test concentrations of fungal spores were added to the air.

5.5 Filter samplers

Electron micrographs of the surfaces of clean, used and washed polycarbonate filters demonstrated a) the efficiency of this type of membrane filter at trapping airborne microorganisms and b) the effectiveness of washing at removing these particles for further analysis. The results achieved compare favourably with those of Palmgren *et al.* (1986) who described the smooth surface of Nuclepore polycarbonate filters and the effectiveness of washing at detaching collected microorganisms. Smooth polycarbonate membrane filters are commonly used throughout the field of bioaerosol research for the collection of airborne microbiological samples (Wang *et al.*, 2001; Durand *et al.*, 2002).

5.6 Towards a standard bioaerosol sampling regime

From the experimental results achieved in this study, it is recommended that the Andersen 6-stage viable sampler be used as standard to effectively and accurately measure bioaerosol concentrations in indoor environments. For completeness of sampling results it may prove useful to perform simultaneous sampling using the AGI-30 liquid impinger, but results between these two samplers have found discrepancies in the collection of fungal cells so this may be unnecessary.

The fact that this work has found that the AGI-30 impinger's collection efficiency for fungal particles is contentious when compared to the Andersen sampler partially supports the findings of Jensen *et al.* (1992). Jensen concluded that either the 6- or 1-stage Andersen sampler or the AGI-30 impinger should be used in circumstances where the sampling conditions are poorly characterised, to

ensure that an air sample representative of the full bioaerosol particle size range is collected.

The results in this study indicate it may also be pertinent to measure total airborne particulates during periods of bioaerosol sampling and this is supported by data from Thatcher & Layton (1995) and Ljungqvist & Reinmüller (2000).

For future research to be performed effectively, it is important to establish a set of standard conditions for the sampling of bioaerosols, both for their collection and detection. This study has shown that the results obtained from bioaerosol monitoring are directly affected by the sampling methods used and so the adoption of a standardised methodology is key to validating the protocols for, and subsequent conclusions of, any future work.

5.7 Measurement of total airborne particulate concentrations using the Negretti LN5 laser monitor

The Negretti LN5 laser sampler has shown to what extremes airborne particulate concentrations fluctuate over time. It can reasonably be assumed that in some situations biological aerosols may show a similar pattern to that observed for total suspended particulates, for example in the vicinity of composting plants, where the microbiological content of total particulate matter is high. There are several situations, however, where it is likely that increases in particulate concentrations will not be reflected in bioaerosol concentrations. These are likely to include urban areas with high levels of soot particles or coastal areas where the

concentrations of salt crystals cause total particulate levels to be high at times when bioaerosols are not (Harrison & Jones, 1995).

Preliminary measurements of total airborne particulate concentrations in an indoor office environment clearly showed measurable fluctuations in particulate concentrations. The results show that the particulate composition of indoor air varies markedly with time. It was observed that the proportion of larger particles i.e. greater than PM10 decreased at night, settling out in the absence of any disturbance. It was also shown that the proportion of total airborne particles made up by the finer particulates, in the range of PM1 – PM2.5, measured at night, was higher as they remain suspended in the air. This is supported by evidence from Leese *et al.* (1997) and Micallef *et al.* (1999). It has been reported that larger particles, such as PM10, often show evidence of being distributed along a vertical concentration gradient as these heavier particles settle out more quickly. Micallef *et al.* (1999) also showed that finer particulates are likely to remain suspended in air for longer and tend to be more evenly distributed by turbulence or mixing. Relating these patterns in particulate concentrations to the time of the day the sample was taken and the occupation status of the room, the observed fluctuations are likely to be caused by environmental disturbances resulting from human activity. This confirms the findings of Micallef *et al.* (1998, 1999) who reported that human activity plays a large role in determining the distribution of airborne particles in the air in confined indoor environments.

5.7.1 Total airborne particulate concentrations and their relationship with measured viable bioaerosol concentrations

It has also been shown that in this study the concentrations of airborne particulates correlate well with measured levels of viable airborne microorganisms in the indoor environments tested. Air sampling results from both the Andersen and Omega samplers followed the same pattern as simultaneously measured particulates. There was evidence of a correlation between specific particle-size concentrations and bioaerosol counts on the stages of the Andersen sampler that correspond to that particle size. Additionally, as well as bioaerosol concentrations, the species composition of samples in relation to particulate data should be examined further in future work. It is important to consider that environmental conditions may affect the shape and size of airborne microorganisms. For example, low relative humidity may result in cells dehydrating and thus shrinking or cells may clump together to be present in the air as one large particle. Both circumstances would result in the bioaerosol of interest being present as a different sized particle than expected which may explain any lack of agreement between particulate data and Andersen sampling results. Further work requires to be done in this area. It would be ideal to develop a model that would allow the prediction of the numbers of airborne microorganisms in an environment from the particulate data measured there. This however, may not be practicable due to the inherent variability seen in particulate and bioaerosol concentrations over time. Future work should explore the predictability of airborne microbial concentrations from high and low background particulate concentrations.

It is important to acknowledge that particulate, and therefore bioaerosol, concentrations are likely to vary markedly over time. The distribution and pattern of behaviour of airborne particulates and bioaerosols measured in this study reinforce the fact that both fluctuate measurably and markedly over time. This observation reinforces the point made in section 4.6, by emphasising that very short sampling times will provide only a small and unrepresentative snapshot of the total particulate picture, where for example, peak exposures may be missed. The moment in time that a sample is taken will have a major influence on the results that are obtained. This should be a major consideration when developing sampling strategies for indoor environments.

5.8 Surface sampling using Dustbuster® vacuum cleaner

There is no standard method for carrying out surface sampling of floors / carpet and different studies have tended to use varying equipment and methodologies. This means that there is a lack of comparability between the results from different studies. Macher (2001a) reviewed a number of studies that examined settled dust and concerns are expressed over the variability in currently used techniques. Researchers have used standardised dust collectors or vacuum cleaners of various types and sizes, to collect samples from various floor areas with various types of floor covering (Lehtonen *et al.*, 1993; Lewis & Breysse, 1998). Despite these conflicts, the surface sampling of office floors carried out in this study was successful, in terms of both the concentrations and species compositions of a possible source of bioaerosols.

5.9 Identification of most common airborne isolates

The bacterial and fungal species isolated and identified during this study are species that are commonly associated with air samples taken from indoor environments. In indoor environments the bacterial content of air is determined largely by the presence of humans and animals, where most of the bacteria isolated are non-infectious (Monn & Koren, 1999). In a domestic environment some of the most common bacterial species are those which have been shed from the human skin or respiratory tract (human-source bacteria). Common indoor bacterial isolates include Gram-positive *Micrococcus*, *Staphylococcus* and *Cornebacteria* (from healthy skin and scalp) and also *Streptococcus* species from nasal and oral secretions (Burge, 1995). The most commonly reported fungal species isolated from indoor air samples are *Penicillium*, *Aspergillus* and *Cladosporium* (Hyvärinen *et al.*, 1993 & 2001; Madelin, 1994; Dotterud *et al.*, 1995; Hunter *et al.*, 1998).

Common fungal species reported in outdoor air include *Aspergillus*, *Cladosporium*, *Penicillium* and *Alternaria* (Verhoeff *et al.*, 1992; Madelin, 1994).

5.10 Acridine orange staining

Ideally, to achieve more accurate staining results, a longer staining time would have been used with each filter. Tests carried out on known spore samples suggested that incubation times of up to an hour would have been more effective. This is likely to be due to the fact that fungal spores are generally constructed to be resistant to environmental stresses such as desiccation, temperature or

chemicals, and it is unlikely that the 5 minute staining time would have been sufficient to allow the acridine orange stain to penetrate the spores' outer coating. Eduard *et al.* (2001) state that errors in identifying fungal spores using fluorescence microscopy are likely to arise from the variations in 'stainability' seen between different spores. Additionally, the dark pigmentation often seen in fungal spores is thought likely to mask any fluorescence resulting from staining (Eduard & Heederik, 1998). Due to constraints on time and equipment, however, it was not feasible in this study to use a staining time of an hour. Only one filter holder unit was available and to use this for 1 hour per filter would have greatly reduced the numbers of samples that could be analysed in one day.

Eduard *et al.* (2001) examined the occurrence of counting errors when using fluorescence microscopy to quantify microorganisms from bioaerosol sampling and concluded that, despite problems with their uptake of stains, the counts of fungal spores were more precise than those of bacterial cells. The small size of bacterial cells is likely to be a major contributory factor to these recognition errors. Additionally, Terzieva *et al.* (1996) suggested that acridine orange counts may overestimate the total number of bacteria in a sample, due to the autofluorescence or non-specific staining of debris matter or cellular components. This is a likely explanation for the excessively high viable bacterial counts obtained from the filter samples analysed using acridine orange staining compared to the expected numbers calculated from concurrent Andersen sampling results. The possibility of dust debris in each sample contributing to counting errors is

fairly high as filter washes were not filtered to remove particulates before they were stained.

The results of this study have shown that fluorescent staining and epifluorescent microscopic examination of bioaerosol samples does not achieve consistent results that can be directly compared to those achieved by the Andersen sampler. Therefore, neither of these two sampling methods allows the accurate prediction of the results that would be obtained using the other.

5.11 Effect of human activity on bioaerosol concentrations in an office

The levels of both airborne and surface dusts (and therefore microorganisms) in an indoor environment are greatly affected by the activities occurring in that area (Thatcher & Layton, 1995; Leese *et al.*, 1997). The results from bioaerosol sampling during normal activity in an office environment showed very definite associations between human activity and increased concentrations of airborne microorganisms.

It has been stated by Madelin (1994) that most fungal particles are present in air as single spores, rather than being carried on larger particles as is commonly seen for bacterial aggregates. Therefore, despite their differences in actual cell size, fungal cells may often be lighter than these common bacterial aggregates. This may explain why, in this study, the fluctuations in particulate concentrations were more accurately reflected by counts on NA. It is possible that heavier bacterial clumps settled out of the still air between periods of disturbance only to be

resuspended when activity resumed. Fungal spores may have remained airborne, due in part to their inherent ability to remain airborne for the purposes of dispersal so that measured concentrations were observed to fluctuate less over time.

These increased levels of dust and bioaerosols associated with human activity and environmental disturbance are likely to be the result of a) shedding from the room occupants, b) activity causing resuspension of settled dust from floor surfaces, or most likely, c) a combination of both of these mechanisms. Lewis & Breysse (1998) discuss the importance of carpeted surfaces as a source of aeroallergens and dust. This is further supported by the analysis of species found in vacuumed dust samples from floor and personal samples, discussed in more detail in Section 5.12.

5.12 Investigating the likely source of indoor bioaerosols in an office environment

The most common species isolated from surface dust samples taken from the head and shoulders of office occupants were Gram-positive rods and cocci. It was hypothesised that there may have been a greater proportion of Gram-negative cells in these samples. This, however, was not the case, with the majority of isolated species being Gram-positive as stated earlier. *Staphylococcus* species are Gram-positive cocci, usually found as clumps of cells. They are commonly pathogenic to humans and, being fairly resistant to desiccation, are likely to survive well in indoor dust. Two of the species isolated in these samples are worth noting. These are *S. epidermidis* and *S. capitis* which are non-pathogenic species normally found

on human skin and mucous membranes (Brock *et al.*, 1994) that produce non-pigmented colonies. *S. aureus*, like *S. epidermidis*, is commonly found living commensally in healthy people on the skin and in the upper respiratory tract (nose and throat). In contrast, however, some strains are capable of causing illness including boils, pneumonia, meningitis and food poisoning. *Bacillus* species are Gram-positive rods that are generally found in soil and are therefore commonly isolated from the air.

Floor dust samples also contained large proportions of *Micrococcus* and *Staphylococcus* species. It should be noted here that very low fungal concentrations were collected from both the floor and personal samples. This result was unexpected, particularly for the samples taken from the floor. This may be an effect of UV exposure through room windows or sample desiccation in floor samples, although this cannot be confirmed from the work carried out in this study.

In conclusion, the bacterial species isolated during surface sampling of room occupants were most likely to have originated from the subjects themselves, rather than being particles that had settled on people from an indoor source. Many of the bacterial species found in the dust samples taken from the office floors were identical to those obtained in the personal samples. It is concluded that these species are present as a result of human presence and activity causing 'shedding' of microorganisms from people into the office environment. This is supported by the findings of Goh *et al.* (2000) who described the flaking of skin cells and

expulsion of microbial particles from the respiratory tract with regard to the numbers and species of bacteria in an indoor environment. Soil-borne species such as *Bacillus* and *Streptomyces* species are likely to be present as a result of ingress from the outdoor environment via windows and doors or transport by people on shoes and/or clothes.

5.13 Effect of human activity on bioaerosol concentrations in domestic accommodation

Bioaerosol concentrations inside a domestic residence, as measured by the Andersen sampler, were clearly influenced by the presence and level of human activity. Vacuuming was shown to have the greatest measurable effect on the concentration of viable airborne microorganisms. The major proportion of each sample was fungal, probably due to the fact that the room being sampled had a severe visible mould problem. Flannigan & Hunter (1988) observed an increase in domestic fungal bioaerosol concentrations, caused by vacuuming, concurring with the results obtained in this study. Work by Lehtonen *et al.* (1993), however, found no effect of vacuuming on airborne fungal concentrations. It has been suggested that vacuuming may only cause an observed increase in bioaerosols if the vacuum cleaner being used lacks an exhaust air filter and so expels very dusty air (Harsh, 1954, Lioy *et al.*, 1999). However, it is also likely that floor dust may be disturbed and resuspended by exhaust air hitting the floor, whether it is filtered or not. Durrell *et al.* (2002) found that, in a study examining airborne concentrations of dust mite allergen, the use of an exhaust air filter did not reduce airborne concentrations of allergen measured during vacuuming. It was regarded

that the observed airborne increases were due to floor dust being raised by the physical action of the vacuuming itself. This interpretation can be applied to the bioaerosol concentrations generated during vacuuming in this study, as the Vax vacuum cleaner used was fitted with a filter at the air exhaust. Lehtonen *et al.* (1993) did find, however, that changing sheets and bedclothes increased the airborne fungal concentrations to 3-5 times the level measured during undisturbed conditions. This value is a little higher than that seen from the sampling results in this study but confirms the relationship between this domestic activity and its effect on measured bioaerosol concentrations.

5.14 The effect of sampling height on the total efficiency of the Andersen microbial sampler

Despite evidence of particulate matter in indoor environments being distributed in the air along a vertical concentration gradient (Micallef *et al.*, 1999), no indication was found in this study for an effect of sampler height on measured bioaerosol concentrations. It is, however, recommended that a sampling height of 1.5 metres, approximately equivalent to the average person's breathing zone, would be most relevant when investigating the effects of bioaerosols on human health. This would ensure, irrespective of the fact that there was no observed difference between heights, that representative samples are taken of those particles likely to be inhaled. Furthermore, the shape and size of a microbial cell will have a significant effect on its aerodynamic properties and hence its ability to remain airborne. This may affect the species that are detected at different sampling heights (Tables 4.4 and 4.5) and possibly also those collected by different

sampling methods. This is an important area which warrants further investigation. Additionally, it is worth considering the effect that the ambient temperature of the environment being sampled may have on bioaerosol (and particulate) distributions and their movement. It is likely that an increased temperature will cause increased air movement and enhanced convection of suspended particles. Higher temperatures may therefore result in a different bioaerosol composition being sampled.

5.15 The effect of incubation temperature on the numbers and proportions of species collected by the Andersen microbial sampler

As previously described in Section 5.1.4, an incubation temperature of 25°C was found to give higher total viable counts on each of four growth media (NA, TSA, MEA and PDA) than those achieved using an incubation temperature of either 30°C or 37°C. Further analysis of sampling data collected in six residential houses examined this relationship in more detail. On NA, no significant difference in the total number of viable microorganisms was found between 25°C and 37°C. Differences were measured for selected species (Table 4.5). In the majority of cases, the species found in greater abundance at a particular temperature were most likely to be from an environment that matched that temperature. Sampling on to MEA however, resulted in a significantly higher total viable cell count at 25°C than at 37°C, concurring with the preliminary results found in this study.

For both growth media, a higher number of fungal species than bacteria were found in significantly greater proportions at 25°C than at 37°C. In fact, for both NA and MEA all fungal species were found at a higher level in samples incubated at 25°C. This difference was not found to be statistically significant in all cases but this result shows evidence that 25°C is likely to be the optimum temperature for successful fungal growth from indoor air samples. This is not unexpected as the most likely fungi to be isolated during indoor bioaerosol sampling are those from environmental sources such as plants or soil (Madelin, 1994).

Further investigation of the effect of incubation temperature on the composition of viable counts should be carried out. Ideally this would involve carrying out the same analyses as described for the data in Section 4.14 but rather than combining the data for all six stages of the Andersen sampler, the results for each plate should be analysed separately, to look for any differences in the species composition per plate for each temperature.

Another important consideration is the likelihood of particular important species being 'missed' from the sampling results due to unsuitable incubation temperatures being used. For instance, when human health effects are of concern in a study, potentially pathogenic species will be of major interest when analysing the results. However, incubation of samples at 37°C is likely to result in a considerable under-estimation of most fungal species – which although unlikely to be pathogenic, have the potential to cause significant health effects (Sections 2.3.5

– 2.3.7). This factor is particularly important and one which should be investigated in further work in this area.

5.16 The relationship between domestic bioaerosol concentrations measured using different sampling methods and the reported health effects experienced by house occupants

It is difficult to find evidence of a definitive relationship between the measured bioaerosol concentrations in this study and the reported health effects of house occupants. A relationship between living in a damp/mouldy home and the respiratory symptoms reported by occupants may not be real. This is likely to be due to the fact that bias may arise from people being aware of the possible relationship between living in a damp house and respiratory morbidity. This awareness may result in a) over-reporting of symptoms by people in damp homes, b) over-reporting of the presence of damp by people with respiratory problems or c) over-reporting of both these factors.

However, the data suggest that there may be a relationship between various symptoms and measured bioaerosol concentrations. This should be investigated further, following the collection of additional samples to allow valid statistical analyses to be carried out.

When examining cases of suspected environmentally-triggered illness, studies tend to combine a simple questionnaire to identify environment-associated symptoms with standard skin tests for allergic sensitivities. To address the lack of

specificity with this approach, Portnoy *et al.* (2000) developed a procedure to attempt to identify indoor sources of sources of bioaerosols. This protocol combined a questionnaire (to gather background information on house type, symptoms, known triggers and contamination sources) with a visual inspection of the property, volumetric air sampling, surface sampling of suspected contaminated surfaces, antigen measurement of house dust and general indoor air quality measurement. Although it was found that in around 80% of homes it was possible to identify a potential source of indoor contamination, it was not possible to show a causal relationship between indoor exposures and health effects.

There were several problems associated with DoH questionnaire data used in this study that may have contributed to a lack of definite results. The questionnaire was administered twice to each householder but often the answers provided to certain questions differed between the two question sessions. This may have been a real phenomenon as perhaps circumstances had changed since the first questionnaire was carried out. However, responses were also found to differ for parameters that should have remained constant throughout the period of questioning. For example, one participant was asked whether they had experienced chest wheeze since the age of 15 – the first response was "yes" and the second "no". Other responses appear confused and do not make logical sense. For instance, a non-smoking woman is regularly exposed to tobacco smoke in the home. The questionnaire data revealed on one hand that there is only one occupant in this household but on the other that 2 people in the house smoke regularly. There is no mechanism to allow for anomalies such as these.

It was also the intention, at the start of this project, to examine endotoxin and glucan concentrations for each house. Data was to be provided from the DoH study but when access was granted to the results it was discovered that valid data only existed for three of the seven houses. This is not a statistically valid number of samples and no suitable analyses were able to be performed on the endotoxin and glucan data. Future work should aim to investigate this further.

5.17 Comparison of reproducibility of results between different sampling methods

Knowing the most common species isolated from an indoor office environment, the results obtained from using different sampling regimes can be analysed with regard to whether they measure comparable bioaerosol concentrations and if they detect the same species or if particular microorganisms are excluded. The efficiencies of different sampling techniques can be compared in terms of whether the sampling method detects the species and numbers of microorganisms that are representative of a particular type of environment.

The AGI-30 all-glass impinger appeared to collect bioaerosol concentrations that were comparable to the results achieved using the Andersen sampler. However, the low fungal measurements recorded by the impinger should be investigated further. It was regarded that the Andersen sampler gave the more representative results of bioaerosol concentration and composition.

The Omega AIRTEST sampler has been shown in several sets of experiments (see results in Sections 4.2, 4.6 and 4.10) to be highly comparable to that of the Andersen sampler. In simultaneous samples taken in both occupational (office) and domestic environments there are no statistically significant differences in the results achieved when sampling on to either NA or MEA, as compared to the Andersen sampler. It can be concluded that the bioaerosol concentrations measured by the Andersen sampler can be used to accurately predict bioaerosol concentrations measured by the Omega sampler and *vice versa*. This is an important attribute to note for further characterisation and evaluation of the Omega AIRTEST, a relatively unknown sampler.

Acridine orange staining of filter samples did not produce results that were comparable to those achieved on either NA or MEA with the Andersen sampler. This lack of agreement raises difficulties when it comes to making direct comparisons between the numbers of bioaerosol particles collected by the two sampling methods.

6 CONCLUSIONS

It has been found that a single sampling method is not suitable for the detection of bioaerosols and that a variety of factors should be considered when designing a bioaerosol sampling strategy. The concentrations of airborne particles, and hence, bioaerosols are subject to intense fluctuations over very short periods of time, most commonly attributable to environmental disturbance caused by human activity. A sampler must be capable of producing representative results from a snapshot sampling period. Too short sampling times or only a few replicate samples may miss peak bioaerosol concentrations or, to the other extreme, detect only the peaks giving an unrepresentative result. The effectiveness of several bioaerosol samplers in indoor environments has been investigated and the most appropriate conditions for their successful use clarified.

The sampling efficiencies of the Andersen 6-stage viable impactor, Omega AIRTEST viable sampler, AGI-30 liquid impinger and filter sampler were evaluated both individually and in comparison with each other. Measurement of indoor particulate concentrations using the LN5 laser monitor and surface sampling of indoor dust were also performed.

The experimental setups that are concluded to allow the collection of representative concentrations of bioaerosols in an indoor environment with low levels of bioaerosol contamination are shown below:

1. Andersen sampler: 10 minute sampling period on to both Nutrient and Malt Extract agars for incubation at 30°C for 72 h.
2. Omega AIRTEST: 140-280 litre samples on to Nutrient and Malt Extract agars for incubation at 30°C for 72 h.
3. AGI-30 liquid impinger: flow rate of 12.5 $l.min^{-1}$ for 25 minutes into 20 ml collection fluid (¼ strength Ringers solution).

It is recommended that all bioaerosol sampling be carried out in parallel with the LN5 laser monitor or equivalent particle counter. These setups will allow the direct comparison of results between the different sample methods.

Although there was no measurable effect of sampling height on bioaerosol concentration, it is recommended that all samples be taken at a height of 1.5 metres, approximately equivalent to the average person's breathing zone. This would be of particular relevance where sampling is being performed to investigate the effects of bioaerosols on human health, ensuring that any samples taken are representative of those particles likely to be inhaled.

It is also important that consideration be given to the high variability of airborne particulate concentrations as shown by the laser sampling results generated throughout this study. For instance when monitoring the indoor bioaerosol

concentrations in a residential environment, it is essential to ensure, as far as possible, that sampling is carried out under normal living conditions that are representative of the everyday activities likely to be occurring in that environment,. There is little point in sampling in an empty home where little disturbance will occur, only for the occupant to return home and cause significant and measurable increases in the particulate and bioaerosol composition of the air.

The establishment of a set of standard conditions for the sampling of bioaerosols is vital for future research to be performed effectively. This study has shown that bioaerosol sampling results are directly affected by the sampling methodology used so the adoption of a standardised protocol is key to valid future work being carried out.

Surface sampling of carpet dust gave an insight into a major reservoir of bioaerosol particles in indoor environments and their susceptibility to the effects of environmental disturbances.

There is a distinct lack of specific dose-response data for exposure to bioaerosols. It was intended that in conjunction with symptom data from another study it would be possible to provide a measure of the number and type of health symptoms likely to be experienced at specific bioaerosol concentrations. Evidence was found of a relationship between higher concentrations of bioaerosols and an increased number of reported symptoms. The data set, however, was too small to allow any statistically significant conclusions to be

made. Further work requires to be done to establish a large dataset that will allow subsequent predictions to be made about the severity of likely health effects according to the measured bioaerosol concentrations recorded for an environment.

7 FUTURE WORK

This work has answered many of the questions set at the outset. However, certain questions still remain unanswered and further investigation is warranted in several areas.

Work needs to be carried out to further characterise the collection efficiency of the AGI-30 impinger, in direct comparison with other samplers. An area of particular importance is its collection efficiency for fungal particles. Ideally testing should be carried out in an experimental environment where known test concentrations of bacterial and/or fungal cells are added to the air, as well as in real-life residential settings.

The relationship between specific particle-size concentrations and bioaerosol counts on the stages of the Andersen sampler that correspond to that particle size needs further characterisation. Ideally a model should be developed to allow the prediction of the numbers (and species) of airborne microorganisms in an environment from the particulate data measured there.

Species distribution at different sampling heights is an important area to be investigated further as the shape and size of different microbial cells influences their ability to remain airborne. Although total bioaerosol concentrations were not seen to be affected by sampling height, the species composition of samples may

vary between different heights. In a similar respect, the effect of incubation temperature on the species composition of cultured samples should be examined further. This is likely to be important with respect to identifying the temperature that is most relevant to finding a relationship between indoor bioaerosol concentrations and health effects.

The data here found evidence of a relationship between health symptoms and measured bioaerosol concentrations. Repeats of the work described here would generate a sufficiently large data set, allowing valid statistical analyses to be carried out and a more comprehensive dose-response relationship for bioaerosol exposure to be established.

The inclusion of endotoxin and glucans monitoring into a bioaerosol sampling regime, as was the original intention, would be of major value to any continuing work in this area.

The results confirm that there is a need to further characterise the samplers commonly used for bioaerosol monitoring.

It is clear from this study that the issues involved are incredibly complex and although some questions remain unanswered, the answers to others have generated important results, some of which need to be pursued further. Ideally, in the future a comprehensive bioaerosol sampling regime will be developed which can allow likely health effects to be predicted accurately.

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